THE REGULATION OF FLOWERING IN *PISUM*

BY

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text.

(J.B. Reid)

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ABBREVIATIONS

LD	long day photoperiod
SD	short day photoperiod
L24	line 24
cv.	cultivar
ED	early developing class
EI	early initiating class
L	late class
LHR	late high response class
$\bar{X}$	mean
S.E.	standard error of the mean
X	probability $<$ 0.05
XX	probability $<$ 0.01
XXX	probability $<$ 0.001
X^2_2	Chi - squared with 2 degrees of freedom
FI	flowering node - the node of first initiated flower counting from the cotyledons as zero
FT	flowering time - time in days from planting to the first open flower
FP	node of first pod counting from the cotyledons as zero
LE	number of leaves expanded
TN	total number of nodes present (including those in the apical bud)
TNE	total number of leaves expanded over the lifespan of the plant
UV	unvernalised
V	vernalised
A/B	refers to a graft with a scion of type A and stock of type B
h	hours
Ptotal	total phytochrome = Pfr + Pr

ABBREVIATIONS (CONTD.)

Pfr	far-red absorbing form of phytochrome
Pr	red absorbing form of phytochrome
R	red light
FR	far-red light
GA_3	gibberellic acid
ABA	abscisic acid
AMO 1618	2 - isopropyl -4- dimethylamino - 5 - methylphenyl - 1 - piperidine - carboxylate methyl chloride
CCC	2 - chloroethyltrimethyl - ammonium chloride
Ethrel	solution of 2 - chloroethylphosphonic acid
SK&F7997	tris - (2-dimethylaminoethyl) - phosphate trihydrochloride
n	number of plants in a particular treatment

SUMMARY

The expression of two genes, *Sn* and *Hr*, which partially control the flowering of peas, was examined in several pure genetic lines. The present study was particularly concerned with the control of the expression of these genes by environmental factors (e.g. light and temperature), the site of action of the genes and their possible mode of action, including simulation of their effects by growth substances. The genotype of the lines used in the study was known at two further loci, *lf* and *e*, the genotypes most frequently used being *lf e sn hr*, *lf e sn Hr* (both early flowering, day neutral types), *lf e Sn hr*, *Lf e Sn hr* (both late flowering, quantitative long day types) and *lf e Sn Hr* (a quantitative long day type showing a substantial flowering delay in warm short days).

Flowering in peas has been postulated to be controlled by the ratio of a flower promotor to a flower inhibitor, the gene *Sn* controlling the production of the inhibitor. In the present study, light from a mixed incandescent-fluorescent source eliminated the difference between genotypes *lf e Sn hr* and *lf e sn hr* in some circumstances. This appears to occur due to a reduction in the activity of the *Sn* gene by light. As little as 4 h darkness is required for the restarting of inhibitor production after the completion of a long photoperiod (20 h). The genotype *lf e Sn Hr* is shown to have a critical photoperiod of between 12 and 14 h at 17.5°C. However, the usefulness of the term 'critical photoperiod' is questioned in plants displaying a quantitative response to photoperiod.

By the use of lights with differing spectral properties, it appears that light controls the expression of the gene *Sn* through two reactions, one in which fairly long durations of far-red light are most effective, and a second

in which short durations of red light are effective and which can be partially reversed by far-red light. The second reaction appears to be mediated by phytochrome, the raising of the Pfr to Pr ratio past a certain point stopping inhibitor production for a certain period (between 6 and 8 h). This switch cannot operate again until over 12 h have elapsed. In the first reaction it is suggested that far-red light is absorbed by some complex in the pathway to inhibitor production resulting in the breakdown of the complex. Consequently, while illuminated with light of this wavelength, inhibitor production cannot occur. During these studies no evidence for the participation of endogenous rhythms in the control of the photoperiod response in peas could be found. It is suggested that under natural conditions the photoperiod response of peas is controlled by the first light reaction, inhibitor production commencing soon after the start of each dark period. The ratio of promotor to inhibitor therefore acts as the timing mechanism and determines whether flowering will occur.

Two sites of the vernalisation response were indicated during grafting experiments with genotypes *lf e sn hr* *lf e Sn hr* and *lf e Sn Hr*, one in the scion (embryonic leaves or apex) and one in the stock (cotyledons). It is suggested that the cotyledon effect is caused by an increase in the ratio of promotor to inhibitor produced in the cotyledons during vernalisation due to a lower temperature coefficient for the formative reactions of the promotor compared to those of the inhibitor. The shoot effect is thought to occur due to a lowering of the threshold of promotor to inhibitor required at the apex for initiation, and possibly also to an alteration in the rate of the aging processes relative to the plastochronic age leading to an earlier (nodewise) rise in the ratio of

promotor to inhibitor. The cotyledon effect in genotype *lf e Sn Hr* gradually becomes more pronounced as the temperature is reduced to 3°C and the length of vernalisation is increased to four weeks. High post-vernalisation temperatures (e.g. 30°C) can reverse this effect. The shoot effect was very stable to normal temperatures but some devernalisation did occur at 30°C, the extent of the devernalisation increasing as the light intensity was lowered. Plants of genotype *lf e Sn Hr* were capable of responding to low temperatures from the time they were developing in the pods on the maternal plant until at least 20 leaves were expanded.

Plants of genotypes *lf e Sn hr*, *lf e Sn Hr* and *Lf e Sn hr* were shown to become more sensitive to long day cycles as they increase in age. This appeared to result from an increase in the ratio of promotor to inhibitor being exported from each leaf as it became older. The gene *Hr* acts in the leaves to reduce the size of this effect. These results support a previous postulation that *Sn* activity decreases with age and that the gene *Hr* specifically reduces this effect. The gene *Hr* also delays the flowering node in decotyledonised *sn* plants under short day conditions but not to any large extent under long days or in intact plants. This would suggest that *Hr* does not operate in the cotyledons and that the gene *sn* is a leaky mutant as suggested previously, unless another mechanism for the action of the gene *Hr* is proposed. The gene *Lf* increases the number of long day cycles required to induce flowering until at least week 5 but does not appear to alter the rate of the aging processes. A discussion of the effects of altering the relationship between chronological and physiological age is given.

The use of L61a (a line of peas having a genotype of

lf e Sn hr and with a penetrance of the *Sn* gene of between 0.4 and 0.6 under normal short day conditions) as a bioassay for compounds affecting flowering is discussed. Treatments known to alter the ratio of promotor to inhibitor such as temperature, light and cotyledon removal are shown to significantly alter the penetrance of the line while altered rates of growth are shown not to affect this variable. However, indirect effects on flowering are still observed as changes in the flowering node of the early and, to a lesser extent, the late classes. Ethrel was the only chemical tested which significantly altered the penetrance of L61a suggesting that this chemical can alter either the ratio of promotor to inhibitor or the threshold of the flowering hormones required for flowering. Further experiments with Ethrel showed that this compound could significantly delay the flowering node of many other lines. However, quite significant differences in the size of this delay were observed but these could not be correlated with the genotypes or phenotypes of the lines used. Many of the effects of Ethrel treatment were similar to those caused by the gene *Sn* but measurement by gas chromotography of the amount of ethylene given off by the genotypes *lf e sn hr* and *lf e Sn hr* under short day conditions showed no measurable differences.

GA_3 and AMO 1618 caused significant alterations in the flowering node of the penetrant L61a plants. GA_3 increased both the flowering node and time to flower initiation under short day conditions but only the flowering node under long day conditions in genotype *Lf e Sn hr*. It was only effective if applied at an early stage of growth. It was suggested that GA_3 slows down the aging processes within the plant. However, the gibberellins do not appear to be

implicated in the action of the gene *Hr*.

A general discussion of the relationship of the present results to other work on the control of flowering in peas is given, along with a model based on both the present results and the previous results of other workers illustrating how the genes and environmental conditions are thought to alter the levels of the flowering hormones. From this model the possible flowering behaviour of presently unreported genotypes under several sets of environmental conditions may be postulated. The model developed for peas is then compared with those for other plants, emphasis being placed on the similarities to models presented for other individual species.

CHAPTER 1

INTRODUCTION

The change from purely vegetative growth to reproductive growth is one of the major developmental changes a plant undergoes. Ample evidence exists (Evans, 1969) to show that, like other developmental sequences, this change-over is accurately determined by the interaction of the genotype of the plant with its environment. The importance of the control by the external environment varies considerably from species to species and even from genotype to genotype within a species (Evans, 1969; Murfet, 1971a). Where the environment has almost no effect (at least within the range of environments normally encountered by the plant) the change-over appears to be controlled primarily by the stage of development of the plant. Whether the stage of development is determined by the sequence of events in the apex independently of the rest of the plant or whether it comes about due to hormonal or metabolic influences arising in other parts of the plant is not clear, although in sunflower the latter would appear to be the case (Wareing and Phillips, 1970). The two most important environmental factors which affect flowering are photoperiod and temperature, although other variables such as light intensity and nutrient status may also play a part. Gassner (1918) working with rye was the first to record the flower promoting effects of low temperatures (vernalisation) while Tournois (1914) working with hops and later Garner and Allard (1920) working with the Maryland Mammoth stain of tobacco and Peking soybeans showed these plants flowered earlier when given short photoperiods (SD plants). Plants flowering earlier as the photoperiod was lengthened (LD plants) had already been observed although the importance of the photoperiod length had not

been established (eg. Klebs, 1913).

The physiological mechanism controlling the change-over from vegetative to reproductive growth is not fully understood for any plant. However, there is a considerable body of evidence suggesting that a graft transmissible hormone(s) plays an important part in some plants, especially those which require a certain photoperiod before the change can occur (Kuijper and Wiersum, 1936; Chailahjan and Yarovoya, 1937). The participation of hormones in the onset of flowering in plants which show little dependence on the environment has also been shown (Murfet, 1973b) as well as in plants which require vernalisation (Melchers, 1937, 1939). It is not clear whether the same hormone(s) is involved in all plants under all environmental conditions, although evidence has been presented to suggest it is similar in some related long day, short day and day neutral species (Chailahjan, 1937; Moshkov 1937; Lang, 1965). However, Melchers (1939) has presented strong evidence from grafting experiments suggesting that the graft transmissible effects due to photoperiod and vernalisation are not similar in Maryland-Mammoth tobacco. Strong evidence of both promotory (Wareing and Phillips, 1970) and inhibitory substances (Guttridge, 1959; Murfet and Reid, 1973) have been found, lending support to the view taken during this work (as well as by Barber (1959) and Evans (1969)) that the mechanism controlling the change from vegetative to floral growth probably varies from species to species although parts of the mechanism may be common to most plants (e.g. the participation of phytochrome in the perception of the photoperiod length in photoperiodic plants). For this reason a study of the control of flowering in a variety of species would seem

worthwhile at least until it is clear which parts of the mechanism are common to all species.

Flowering in *Pisum* has been studied extensively for over a century (e.g. Mendel, 1865) and the progress made has been reviewed twice within the last seven years (Haupt,1969; Murfet,1976). For this reason only brief comment will be made here on the present state of our knowledge of this field, emphasis being placed on opinions held by various groups working in the field and the gaps in our overall knowledge of flowering in peas.

Several phenotypic classes of peas exist (Marx,1968; Murfet,1971a) and consequently a knowledge of the genetic differences between these types is required before a full understanding of the physiology of flowering can be obtained. The results obtained by the geneticists can be divided into two groups; one group using biometrical techniques on plants grown in the field (e.g. Clay, 1935; Rowlands, 1964; Watts et al., 1970; Snoad and Arthur, 1973a, 1973b) found that flowering appeared to be controlled by simple additive polygenic systems although some dominance for both late (Rowland,1964) and early flowering (Snoad and Arthur, 1973b)has been reported. The second group used controlled environments (photoperiod being the most important) to accentuate the differences between the phenotypic classes and used Mendelian techniques to try and separate out individual genes (e.g. Barber, 1959; Marx, 1968, 1969; Murfet, 1971a, 1971b, 1973a, 1975). This second approach has led Murfet to suggest that there are at least four major loci, *lf, e, sn and hr,* concerned with the control of flowering in peas. As well, several polygenic

systems exist from which further major loci may be isolated under other environmental conditions.

The study of the physiology of flowering in peas has been restricted largely to investigations of the early and late cultivars (corresponding to Murfet's ED and L phenotypes respectively). Three major schools of thought exist on how this difference is maintained. The German school, led by Haupt (1969) and Köhler (1965), suggests that the early cultivars produce a flower promotor which is absent in late cultivars. An Australian group (Paton and Barber, 1955 ; Barber, 1959; Sprent and Barber, 1957; Paton, 1967, 1968, 1969, 1971; Amos and Crowden, 1969) favour the opposite view, namely, that late cultivars possess a flower inhibitor (whose production is controlled by the gene *Sn*) which is not present in early cultivars. Murfet (1971b, 1971c) suggests that both a promotor and an inhibitor exist, the late cultivars containing larger quantities of the inhibitor.

Murfet (1971b, 1971c, 1973a, 1975) has extended his experiments to include all 5 of his phenotypic classes and has postulated mechanisms by which the alleles at his 4 major loci may operate. Since my work used his phenotypic classification and lines, a brief description of his conclusions is required. His five phenotypic classes are:-

ED (early developing): Flowering node and time are unaffected by photoperiod and are both early under short days. Pure ED varieties normally flower in the range of nodes 9 to 12.

EI (early initiating): Flowering node is unaffected by photoperiod and is normally between nodes 9 and 12 for pure varieties. Flowering time is however early in long phot-

periods but late under short photoperiods because of retarded development or abortion of the first flower buds.

L (late): Flowering node and time are both delayed by short photoperiods. The flowering node is normally 13-18 in long photoperiods and 20-35 in short photoperiods.

LHR (late high response): Flowering node and time are similar to L plants in long photoperiods but are both markedly delayed by short photoperiods. They normally flower above node 35 under warm short photoperiods.

VEI (very early initiating): Flowering node is very early, usually in the range of 5 to 8.

The genotype at the four loci, *lf, e, sn and hr*, determines to which phenotypic class a particular plant belongs. The genotype *lf e sn hr* is ED. Addition of *Sn* creates an L-type. *E* is epistatic to *Sn* in terms of flowering node and *lf E Sn hr* is phenotypically EI. *Lf* is epistatic to *E* and genotype *Lf E Sn hr* is again L-type. The combination of *Sn Hr* confers the ability to show a very large response to photoperiod and genotypes *lf e Sn Hr* and *Lf E/e Sn Hr* are phenotypically LHR. Genotypes *Lf e sn hr*, *lf E sn hr* and *lf e sn Hr* are basically ED, *Hr* conferring some EI tendencies and *Lf* raising the flowering node. Two further alleles, lf^a and Lf^d are proposed for the *Lf* locus, giving a dominance order of $Lf^d \rangle Lf \rangle lf \rangle lf^a$ with a coincident decrease in the flower delaying ability.

From grafting experiments Murfet (1971c, 1973a and 1975) suggests that *Sn* produces a flower inhibitor in the cotyledons and shoot, this being favoured by short photoperiods; that *E* lowers the level of inhibitor in the cotyledons; that as the series lf^a, *lf*, *Lf* and Lf^d increases the apical sensitivity to inhibitor is increased and that *Hr* delays the apparent effect of aging on *Sn*. As well as these four major genes,

polygenic modifier systems occur which may vary the flowering behaviour within or even between these phenotypic groupings (e.g. penetrance modifiers (Murfet, 1971b)).

The work presented in this thesis attempts to extend the understanding of the mechanisms of action of the genes *Sn* and *Hr*, primarily by a study of the four genotypes *lf e sn hr*, *lf e Sn hr*, *lf e sn Hr* and *lf e Sn Hr*. Since *Sn* confers an ability to respond to both photoperiod and vernalisation (Murfet and Reid, 1974) the effect of these two environmental variables as regulators of the activity of *Sn* was studied. Grafting experiments were also performed to gain an insight into the site of action of the genes and the environmental variables. In addition, experiments were carried out to try to determine the possible biochemical actions of these genes. Details of the aims and reasons behind a particular series of experiments are given in the introductions to the subsequent chapters.

CHAPTER 2

GENERAL MATERIALS AND METHODS

General reference is made to the growing techniques and experimental procedures which were used in more than one of the chapters. Where specific techniques were used these are referred to in the relevant experimental chapter.

Growing Conditions

The plants (unless otherwise noted) were grown in either 2.7kg cans, 14cm slimline pots or plastic tote boxes in a 1:1 mixture of new vermiculite and 6mm dolerite chips and treated with nutrient solution (a modified Hoaglands solution until June 1974 and Aquasol thereafter) twice a week. The change in nutrient type does not appear to have affected the results in any significant way. The plants were watered once a day. The seeds were normally germinated 2-3cm below the surface of the growth medium. Seeds were nicked in experiments involving the gene A, vernalisation or chemical treatment to the surface of the dry testa in order to facilitate rapid and even germination. This is especially important if flowering time (FT) is to be recorded. These conditions allowed the survival of 95-100% of the untreated seeds.

The plants were grown in either growth cabinets or glasshouses. Our controlled environment facilities can be divided into three types. Firstly, there are facilities to allow large scale growth of plants under controlled photoperiod conditions. In this facility the plants were grown in a glasshouse on trucks 9m long with supports for plant 2m tall. These trucks can move in and out of compartments each day and were normally set to give an 8h photoperiod of natural light (SD).

The minimum night temperature was 15°C the maximum being occasionally as high as 23°C. However, since March, 1974 refrigeration units have allowed the temperatures in these compartments to be maintained permanently between 15 and 18°C. The trucks could remain out of the compartments and the natural photoperiod could be extended up to 24 h of light each day (LD) by supplying light at an intensity of 1600 lux at plant height from a mixed fluorescent-incandescent source. The night temperature in the long day area was maintained above 14°C. The day temperatures were extremely variable in this facility as the glasshouse is cooled only by vents. These were normally set to open at 23°C. Seasonal variation in temperature and light intensity could therefore not be eliminated whilst using this facility.

Secondly, four 3m x 2m x 1.2m cabinets were available. Both temperature and photoperiod can be accurately controlled. Light is supplied from a source consisting of 360 watts of fluorescent tubes and 160 watts of incandescent bulbs. This source can be raised and lowered and allows ample light for active growth. The lights were normally set to give 23,500 lux at 10cm above the growth medium. This facility was used for controlled photoperiod experiments and to allow for treatments of plants with red (R) and far — red light (FR). In a number of experiments these cabinets were used only during the 16h night, plants being moved to the glasshouse for 8h of natural light.

Thirdly, four small cabinets with both controlled photoperiod and thermoperiod were available for controlled growth during early development. These cabinets are only

25cm high and consequently growth of dwarf, intact plants beyond node 12 was not possible.

Characters Recorded

The principle character recorded was the flowering node (FI) and it is taken in this work as the node at which the first flower bud is initiated, counting from the cotyledons as zero. Data were recorded from the main shoots only. Under the growing conditions used laterals rarely developed on plants exposed to LD but did develop from the lower nodes on plants exposed to SD. Laterals that did develop were regularly removed. The flowering node was taken as representing the developmental stage at which the balance of the flowering hormones first shifted in favour of flowering. It is a robust character which does not appear readily altered by small fluctuations in the vigour of plants and is therefore a very useful character. This change to the reproductive phase need not be final, reversion to the vegetative phase occuring frequently in some treatments and this was also recorded.

The flowering time (FT) (number of days from sowing to the opening of the first flower) was also recorded in many experiments and varies widely between genotypes. It depends on the flowering node, the rate of development of the flower buds and the rate of leaf expansion and consequently varies widely between treatments which affect the vegetative growth of the plant. For this reason care needs to be used in interpreting alterations of FT as changes in the flowering process.

Other measures of the flowering response are occasionally mentioned. They are the node at which the first open flower

occurs (FD), the node at which the first pod is formed (FP) and the time at which initiation occurs. Only the last term warrants further explanation since it has caused considerable controversy. Collins and Wilson (1974a, 1974b) suggest it is the best measure of the change from vegetative to reproductive growth. The author feels that this character is not as reliable a guide as the flowering node since it varies considerably with the growth of the plant in a way which does not reflect the hormonal situation within the plant. For example, vigorous self-grafts usually show little alteration of the flowering node but the time to initiation may be substantially altered. However, even though the character is also difficult to score, since the plants must be dissected, it was still used in some experiments concerned with the aging processes in order to try and differentiate between direct and indirect effects on the flowering process.

The stage of development of plants was estimated largely by recording the number of leaves expanded (LE) at a particular time. This character was normally measured by tagging the last open leaf at some time prior to flowering. If more accurate estimates of LE were required the decimal system of Maurer et al. (1966) was used. A better estimate of the developmental stage of plants is given by finding the total number of nodes (TN) a plant possesses at a particular time. By dividing the change in LE or TN values by the length of time between successive measurements an estimate of the rate of growth of the plants can be found. Since it is impractical to obtain TN values in all experiments due to time and space requirements, a typical set of data showing the values of LE and TN at various times throughout the growth of a group of L63 plants grown in an 8h

photoperiod on the trucks is shown in fig. 2.1. The number of nodes in the apex (NA = TN - LE) is not constant throughout the growth of the plant, increasing in these data from 6.5 at day 1 to 12 at day 23 and above. Different NA values can also occur between different treatments possessing the same LE value (Reid and Murfet, 1974a). For this reason, although the graph is a useful guide to the TN value of intact plants at a particular LE, when exact values of TN were required samples of experimental plants have been dissected. The relationship of both TN and LE to chronological age is very variable and depends markedly on the temperature and the amount of light reaching the plant. Comment will be made in chapter 5 about the effect of the chronological age and stage of physiological development of the plant on the flowering process. Neither TN nor LE plotted against chronological age fits a perfect linear regression (fig.2.1.) possibly because small environmental differences occurred (e.g. temperature, light intensity) over the period of the experiment and secondly, a slight reduction in growth rate was observed between days 20 and 30 possibly due to the senescence of the cotyledons. Under the conditions used the length of one plastochron was 2.0 days.

Lines

The pure lines 53, 58, 60, 61a, 63, 64 and 68 were developed from the crossing programme at Hobart. Lines 59, 24 and 51y are single plant selections from commercial Massey, Greenfeast and Vinco seed respectively. Lines 7 and 8 were obtained from R. Lamm of Alnarp. The genotypes, phenotypes and physiological behaviour of these lines have been extensively reported by Murfet (1967, 1971a, 1971b, 1971c, 1973a, 1973b, 1975). The

phenotypes and genotypes at four loci which affect flowering are tabulated for these lines in table 2.1. It should be noted that although several lines possess the same major flowering genes they differ in their polygenic backgrounds (e.g. L53 and L61a; L68 and L58).

Vernalisation

This was carried out by planting the seeds in the usual manner and then placing the plants at the prescribed age in the cold room at between 2° and 4°C for the required period. If this was done as soon as planting was completed the plants grew at a rate where 1 day of normal SD conditions was equivalent to 1 week of vernalisation. In experiments in which differing lengths of vernalisation or both vernalised and unvernalised plants were required, the planting times were normally staggered so that at the completion of vernalisation all plants would be at approximately the same stage of development.

Grafting and Decotyledonisation

Grafts between young scions and young stocks were done using the technique described by Murfet (1971c). Briefly, the seeds are germinated 3 cm below the surface of wet vermiculite at the required temperature. The grafts were made on days 4,5 or 6 (for unvernalised plants) when the plumules were still hooked and 1 - 2 cm long. For vernalised plants grafting was done at the completion of vernalisation, the plumules again being 1 - 2 cm long. The stock plant was decapitated just below the apical hook and a small band of rubber placed over the stump. A slit was then made with a scalpel in the stump. The scion plant

was decapitated just above the cotyledonary junctions and the epicotyl trimmed to form a wedge. This scion was then slipped into the slit in the stock. A firm union normally formed within 24 h. Any laterals from the cotyledonary axils were regularly excised. The grafts could be divided into two groups, vigorous grafts which began active growth within 10 days of grafting and attained a similar growth rate to that of the intact controls thereafter and slow grafts which did not commence active growth for 2 - 4 weeks after grafting and looked similar to decotyledonised plants. These two classes of grafts are dealt with separately in the text due to differences in their flowering behaviour in some circumstances (Paton, 1969; Murfet, 1971c). Cotyledon removal was performed at the same developmental stage at which grafting was performed in the young plants. Grafts using older plants were also performed. The technique used was similar to that described above for young plants, the scion being cut at the base of the required internode and the stock at the top of the internode. In experiments using plants more than a week old at the time of grafting, the grafts were covered with a plastic bag for up to one month in order to reduce the transpiration rate.

Chemical Treatments

Treatment was normally made by either dissolving the chemical at the prescribed concentration in water and placing approximately 20 ml of the solution in a glass Petri dish containing a layer of cotton wool and 4 seeds with their testae nicked, or by placing 10 µl of ethanol containing the correct amount of chemical on the nicked side of the dry seed and allowing the ethanol to evaporate. In the first method the plants were transplanted onto the surface of the growing medium

when the radicals were 1.5 - 3 cm long (days 3-5), while in the second method the seeds were placed 3 cm below the surface of the wet growth medium and allowed to germinate without further watering for the first 48 h.

Table 2.1 The phenotypes and genotypes at four loci controlling flowering are shown for the lines used during the present study.

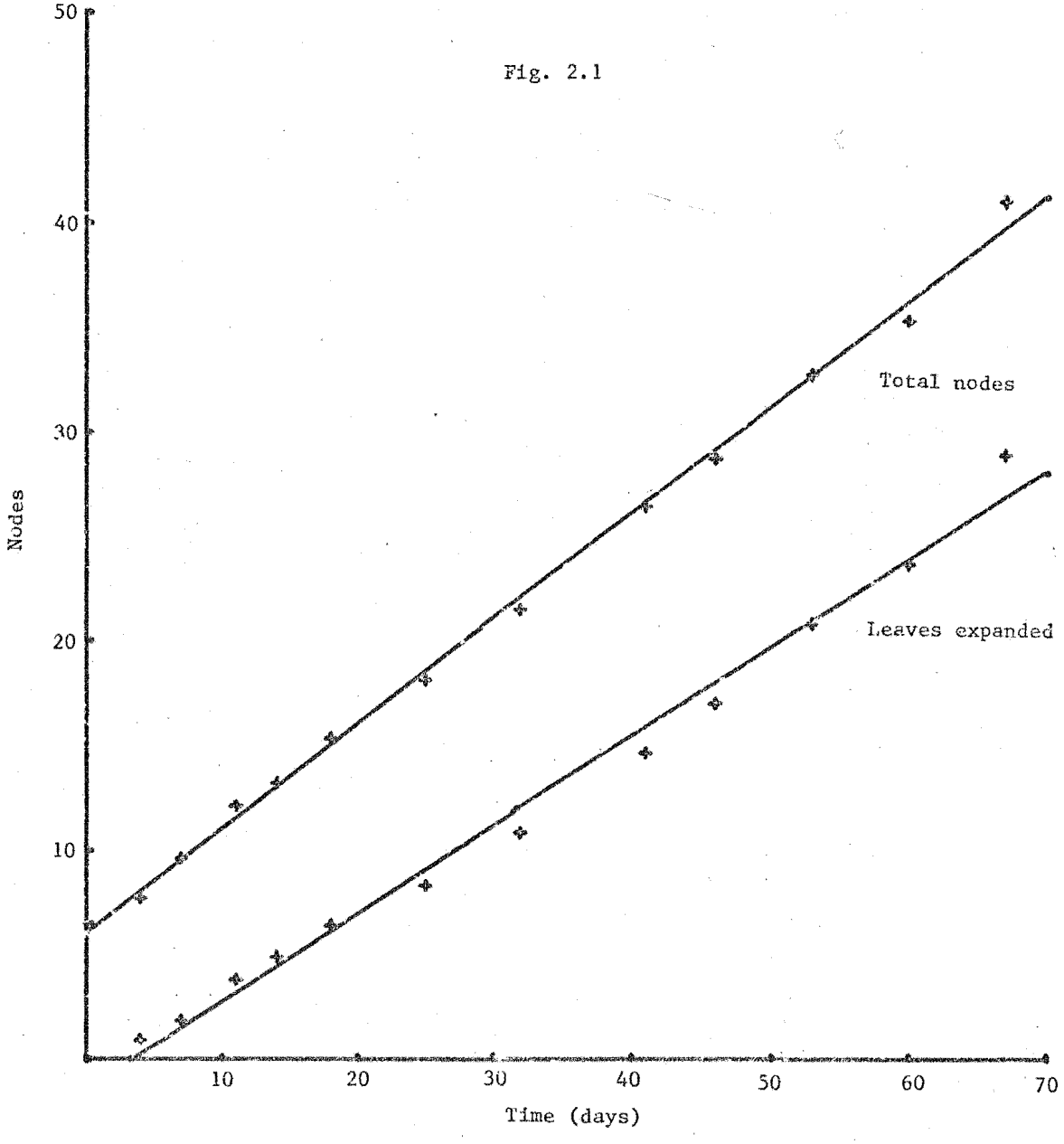
LINE NUMBER	PHENOTYPE	GENOTYPE
7	VEI	*lf*^{a} *E Sn hr*
8	EI	*lf E Sn hr*
24	L	*Lf e Sn hr*
51y	ED	*lf E sn Hr**
53	L	*lf e Sn hr*
58	ED	*lf e sn hr*
59	ED	*lf E sn hr*
60	EI	*lf E Sn hr*
61a	EI/L	*lf e Sn hr*+
63	LHR	*lf e Sn Hr*
64	ED ø	*lf e sn Hr*
68	ED	*lf e sn hr*

*This line may be heterogeneous at the *E* locus.

+This line possesses a polygenic background which lowers the penetrance of *Sn* to approximately 0.5 under normal SD conditions.

ø This line possesses distinct EI tendencies.

Fig. 2.1


50
40
30
20
10
Nodes
Total nodes
Leaves expanded
10
20
30
40
50
60
70
Time (days)

CHAPTER 3

LIGHT

Determination of the Critical Photoperiod and the Sensitivity of Genotypes *lf e Sn hr, Lf e Sn hr and lf e Sn Hr* to Inductive Cycles at Various Ages.

INTRODUCTION

Very little detailed data are available in the literature regarding the 'critical' photoperiod in peas. Barber (1959), Paton (1968), Marx (1969) and Wellensiek (1969a,1973b) all provide data examining the action of three widely separated photoperiods on both early and late cultivars of peas. The latter two authors also appear to have included cultivars which are capable of a very high response to photoperiod but the results are incomplete, many treatments not flowering under the experimental conditions used. The results from late cultivars show a gradual lowering of the flowering node as the photoperiod length is increased, the delays becoming larger as the photoperiod length is decreased towards 8h. These results are typical of the responses shown by quantitative LD plants. Since genotypes which are capable of a high response to photoperiod (LHR types) have been the least studied and they tend towards qualitative LD plants it was decided to study the flowering behaviour of the LHR genotype *lf e Sn Hr* under a series of photoperiods from 12 to 24h.

Murfet (1971c) has suggested that the reason the genotype *lf e Sn hr* is a quantitative LD plant is that the activity of the gene *Sn* decreases with age. The gene *Hr* is a modifier of *Sn* which possibly acts by reducing this aging effect (Murfet, 1973a). If the above hypothesis is correct it would be expected that the gene *Hr* and the age of the plant would affect the sensitivity of peas to inductive cycles.

To examine this question the genotypes *lf e Sn hr*, *lf e Sn Hr* and *Lf e Sn hr* were exposed to various numbers of long days. The last genotype was included to see whether the third major latening gene, *Lf*, affected the aging process and whether its activity extended into the late flowering region.

MATERIALS AND METHODS

Determination of the number of long day cycles required for flowering:

The results (fig. 3.1) come from two separate experiments due to the limitations of space under controlled photoperiod conditions. The seeds were germinated 3 cm below the surface of the growing medium and had their cotyledons exposed to the 8h photoperiod after one week. Each week 36 plants of each genotype were tranferred from short days to continuous light. They were divided into three groups of twelve, each group being given a different number of LD cycles. One LD cycle extended from 8.30 a.m. one day until 4.30 p.m. the following day (i.e. 32h of continuous light). Two or more cycles had this basic 32h stretch of light plus additional multiples of 24h light. The light source used to extend the natural photoperiod was a mixed fluorescent-incandescent source with an intensity of 1600 lux at plant height. From preliminary experiments it was possible to estimate the number of LD cycles to be given at each age so that three groups could be expected to span the region from below 50% flowering to 100% flowering. This was successfully done in 17 out of 21 cases. The point plotted in fig. 3.1 is the number of long days required to induce either 50% or 100% flowering, the values plotted being interpolated from the raw data. The actual number of cycles required for 100% flowering is contained in Appendix 1. The number of leaves expanded was recorded at the time of transfer of plants from

short days to continuous light. From these data regressions of leaves expanded on time showed that the rate of leaf expansion was between 1.94 and 2.63 leaves per week for all three lines in both experiments.

In the first experiment only L63 plants (genotype *lf e Sn Hr*) were tested and the results from this experiment form the graph for L63 from week three onwards. In the second experiment lines 53 *(lf e Sn hr)* and 24 *(Lf e Sn hr)* were tested as well as a sample of L63 plants to be tested at 2,4 and 6 weeks of age. At 4 and 6 weeks the results for L63 plants were the same in the two experiments, but at 2 weeks a small difference occured, the results from the second experiment being used in the drawing of the figure since these were more closely comparable with the results for lines 53 and 24. Control plants maintained continuously under either short or long photoperiods were also grown in each experiment. The first experiment was conducted from June till September of 1973 and the second over the same period in 1974. Consequently, mean temperatures were approximately the same, the range being from 15 to 19°C for the night and from 14 to 30°C for the day temperatures.

Determination of the critical photoperiod in genotype *lf e Sn Hr*:

L63 plants were exposed to either 12, 13, 14, 15, 16, 18, 20 or 24h of light each day from the time their shoots emerged through the surface of the growing medium until day 58. They were grown in the large cabinets, the light source being a mixed incandescent-fluorescent source giving an intensity of 23,500 lux at plant height. Two runs were necessary due to the

lack of cabinets, the photoperiods 12,13,14 and 15h being given in the first run and the photoperiods 16, 18, 20 and 24h in the second. If the plants had not flowered by day 58 they were transferred to SD conditions on the trucks for about 5 weeks before being transferred to a long photoperiod on the apron. The results are contained in fig. 3.2.

RESULTS

From fig. 3.1 it can be seen that all three lines tested show an increased sensitivity to LD cycles as they increase in age. The range is from a requirement of over 10 LD cycles to induce flowering in 50% of L24 plants after one week to one LD cycle being sufficient to cause 100% flowering in L53 plants after five weeks. This effect is most easily considered as a decrease in the expression of the gene *Sn* as the plant ages. However, the graphs for lines 24, 53 and 63 are not identical and it is suggested that these differences largely reflect the action of the genes *Lf* and *Hr*. It should be noted, however, that the lines possess different genetic backgrounds and these may also account for some of the observed differences. The action of gene *Lf* is indicated by comparing the curves for lines 53 *(lf e Sn hr)* and 24 *(Lf e Sn hr)* L24 requires significantly (at the 0.01 level) more LD cycles to induce flowering than does L53 which is consistent with the suggestion that *Lf* increases the promotor to inhibitor ratio required for flowering (Murfet, 1971b,c). This effect is evident up until at least week 5 (approximately 21 nodes had been laid down at this stage) indicating that *Lf* is still active in the late flowering region (flowering nodes between 17 and 35). After week 6 both L24 and L53 were fully induced by one LD cycle. When maintained under SD conditions lines 53 and 24 did not flower

at significantly different nodes in this experiment although usually (Murfet, 1971a, 1971c; Murfet and Reid, 1974) L24 flowers slightly later than L53. These results suggest that *Lf* does not influence the relationship between plant age and the activity of the gene *Sn*.

The effect of gene *Hr* can be seen by comparing the curves for lines 53 and 63 *(lf e Sn Hr)*. There appears to be no significant difference in the sensitivity of lines 63 and 53 to LD cycles over the first three weeks of growth indicating that gene *Hr* has little or no effect until about the fourth week. From this time onwards the curves for lines 63 and 53 are markedly different, all line 53 plants flowering by the seventh week(even under continuous short days) while 67% of L 63 plants had not flowered on transferral to long days after 14 weeks' growth. This evidence supports the suggestion that the gene *Hr* reduces the effect of age on the gene *Sn*, allowing a fairly stable (but inhibitory) promotor to inhibitor ratio to exist (Murfet, 1973a). As can be seen from fig. 3.1, the genotype *lf e Sn Hr* is therefore suited to work on the control of the flowering process since it is very sensitive to changes in the photoperiod over at least a six week period.

L53 plants seem to be able to 'remember' that they were exposed to a non-inductive number of LD cycles given up to four weeks previously. This is shown by the fact that plants not induced by a small number of LD cycles during treatment at weeks 2,3 and 4 flowered on average 1.96 nodes earlier (significant at the 0.001 level) than plants given continuous short days. In L24 plants this difference is 1.02 nodes (not significant). These results suggest that there is quite a slow turnover of the flower-

ing hormones.

In successfully induced L63 plants the difference between the flowering node (FI) and the number of leaves expanded (LE) at the start of photo-induction decreased as the number of LD cycles increased. For example, in the eight week treatments, one LD cycle caused 75% of the plants to flower and the difference between FI and LE was 14.11 $\pm$ .31 nodes. Two LD cycles caused 100% flowering and the difference between FI and LE was reduced by 1.6 nodes to 12.50 $\pm$ .17 (significant at 0.001 level). Therefore, although one LD cycle is capable of inducing some plants it does not cause as rapid a change in the ratio of promotor to inhibitor at the apex as does two LD cycles. In treatments that caused incomplete or just complete flowering in L63 plants vegetative reversion was common, reaching 45% in one treatment. Barber (1959) showed similar vegetative reversion in the late cultivar Zelka. This illustrates that on transferral back to short days the ratio of promotor to inhibitor may again decrease sufficiently to cause some plants to revert to the vegetative state. Along with the fact that inhibitor production can recommence with the onset of each dark period (Murfet and Reid, 1974) these data support the suggestion by Barber that photoperiodic induction is reversible in late cultivars of peas. This situation appears similar to that occuring in *Glycine max* (Lang, 1965) but is distinctly different from that reported for the SD plants *Xanthium* and *Perilla* in which a leaf, once induced, appears to remain so almost indefinitely (Zeevaart, 1958).

Amongst plants induced to flower by LD cycles considerable variation occurred in the stage of floral development reached. Although this variation was not specifically analysed

since the vigour of the plant had a large effect on this development, it did appear that those plants with the most developed flower buds had been exposed to the largest number of LD cycles. None of the exposures given was sufficient to cause the plants to senesce, a state which normally occurs rapidly after flowering in L63 plants exposed to continuous light.

L63 plants show a continuously increasing delay in the flowering node as the photoperiod is shortened from 24h to 14h (fig. 3.2). This response is essentially similar to that observed in many quantitative LD plants including the late pea cultivar Greenfeast (Paton, 1969). However, in photoperiods with less than 16h light the delay in L63 is much larger than that observed in Greenfeast. Under a 13h photoperiod only 36% of L63 plants had been induced to flower before the treatment was stopped after 58 days (at which time $25.74 \pm .23$ leaves were expanded) while under a 12h photoperiod no plants were induced to flower by the treatment, induction not occuring until after transfer to a long photoperiod following the completion of 5 weeks of SD conditions. These results suggest no point of discontinuity which is usually associated with the critical photoperiod in many other plants (e.g. *Xanthium strumarium*, *Pharbitis nil*, *Glycine max*, *Sinapis alba* (Salisbury, 1969; Takimoto, 1969; Hamner, 1940; Bernier, 1969)). Instead for initiation to occur the plants require only a longer period of exposure as the photoperiod is decreased. This view is reinforced by the observation that under similar conditions L63 plants exposed to a 12h photoperiod will flower provided the length of exposure is long enough (table 3.9). The response of L63 to photoperiod is therefore similar to that observed in the CERES and Ba 6139-7 strains of *Lolium temulentum* (Evans, 1958,

1960a; Peterson and Bendixen, 1963). It has not been specifically determined whether the duration of exposure or the age at which the plants are exposed to a particular photoperiod is of prime importance although the results relating to aging would suggest that the latter is probably the case.

DISCUSSION

The decrease in the number of LD cycles required to induce lines 63, 53 and 24 as the age of the plant increases is similar to the decrease shown in the long day plants *Lolium temulentum* (Evans, 1960b) and *Sinapis alba* (Bernier, 1963). In peas at least three major genes influence this sensitivity to long days. *Sn* confers the ability to respond to photoperiod (Barber, 1959; Murfet, 1971a) and from the present data appears to decrease in effect as the plant ages. *Hr* blocks this decreased effect with age from about week 4 while *Lf* increases the number of LD cycles required up till at least week 5. The decreasing effect of the *Sn* gene as the plant ages may be explained either by a decrease in the activity of this gene in the leaves where it would be expected that a gene under photoperiod control would operate (Murfet, 1971b), by a drop in the level of promotor, or by a lowering of the ratio of promotor to inhibitor required at the apex for flowering. In the results the first of these alternatives has been favoured. If the effect is in the leaves each individual leaf could age (i.e. become more promotory with age) or the higher the node number of a leaf the more promotory it may be. Experiments designed to answer these questions are contained in Chapter 5. The site of action of the gene *Hr* I anticipate to be the same as the site of the aging of gene *Sn* since *Hr* is a specific modifier of *Sn*, having little effect in the absence

of *Sn* (Murfet, 1973a). Gene *Lf* is effective with or without *Sn* (Murfet and Reid, 1974) and although its site of action is in the shoot (Murfet, 1971c) it is not known whether the leaves or apex are primarily affected. However, the suggestion that *Lf* affects the ratio of promotor to inhibitor required at the apex for flowering (Murfet, 1971b,c) is consistent with the present data.

Jacobs (1972) has shown that the minimum number of inductive cycles required to induce complete flowering in *Xanthium* and *Perilla* is the same as the number of days in a plastochron and has suggested that this phenomenon is fairly widespread in other species. In the present experiments the plastochron for line 63 plants was 64h (from the regression of the number of leaves expanded on time), while one LD cycle (32h of light) was sufficient to induce 100% of plants after 10 weeks' growth indicating that peas, like *Lolium temulentum* (Evans, 1960b) and *Sinapis alba* (Bernier, 1963, 1966) do not fit into the group of plants described by Jacobs.

The critical photoperiod for long day plants is widely accepted as the minimum photoperiod in a 24h cycle capable of inducing flowering. Sometimes it is taken as the photoperiod capable of inducing 50% of plants (Vince-Prue, 1975). Whichever definition is used, the critical photoperiod in 58 day old L63 plants grown at 17.5^{o}C is between 12 and 14h (fig. 3.2). The critical photoperiod used in this sense would be markedly altered by the age of the plants used and probably the growing temperature. Wellensiek (1973b) has suggested that the term 'critical photoperiod' should not be used in relation to peas and suggested the term 'critical duration of exposure' as a suitable replace-

ment in plants showing a quantitative response to photoperiod. This term is defined as the length of the photoperiod in hours multiplied by the number of nodes to the first flower, it being suggested that this figure should be a constant, at least over an intermediate range of photoperiods (14-17.5h). When applied to the present results this was not found to occur, wide variation in the results occuring over even a restricted range of photoperiods, suggesting that this term is not of general application even to other genotypes of peas. Murfet (1976) has suggested that the critical photoperiod should be redefined in species which do not show a qualitative response as the photoperiod at which the rate of change in the response curve is at a maximum. It would appear that for this definition to be of use the type of equation to be fitted to the curve should be defined, since a meaningful second differential is essential. Subjectively this point would appear to be between 16 and 17h for the present data. However, I feel there is no need to postulate an underlying biochemical discontinuity to account for this apparent change. I feel that the amount of inhibitor produced under the different photoperiods used in this experiment is directly proportional to the length of the dark period and that this amount gradually decreases as the plants age. Consequently, if the ratio of promotor to inhibitor required for flowering is constant the flowering node would be expected to rise more rapidly as the photoperiod is decreased since the threshold would be approached more slowly as the photoperiod becomes shorter. The author agrees with Wellensiek (1973b) that the term 'critical photoperiod' is an unsuitable term when applied to plants which show a quantitative response to photoperiod, especially where the response can be markedly affected by the growing temperature and age of the plants. However, any attempt at redefinition of the term would appear

impractical, it being considered more important to carefully define the genotype, temperature, age of plants, etc. from which the results were obtained.

The Action of the Gene *Sn* and the Control of its Activity by Light.

INTRODUCTION

There has been considerable controversy over whether flowering in peas is controlled by a flower inhibitor, flower promotor or both. Recently Murfet (1971b,c) has suggested that both occur, Murfet and Reid (1973) providing strong evidence to suggest that the gene *Sn* controls the level of a graft-transmissible flower inhibitor. Whether the level of inhibitor is controlled by the photoperiod as suggested by Barber (1959) and Murfet (1971c) or whether inhibitor production just allows a differential rate of production of a flower promotor to be observed (Amos, 1974) is not known and has been examined using grafting experiments between genotypes *lf e sn hr* and *lf e Sn hr*. The results suggested the former view and consequently the question of whether the level of inhibitor was controlled by differential production (Murfet, 1971c) or breakdown (Barber, 1959) was investigated.

Murfet and Reid (1973) have obtained some evidence that *Sn* is totally inactivated by continuous light. This evidence is apparently contradicted by the fact that under continuous light plants of genotype *lf e Sn hr* are normally 4-6 nodes later than plants of genotype *lf e sn hr* when planted with their cotyledons buried. However, this difference could arise from *Sn* activity in the cotyledons which are normally buried and therefore in the dark. This question has been specifically checked by exposing plants of genotypes *lf e sn hr*, *lf e Sn hr* and *lf e Sn Hr* to continuous light from the start of germination. The length of the dark period required before the *Sn* activity is observed was also examined using 16, 18 and 20h photoperiods

as well as continuous light. The only data available on this point at present are those of Paton (1968) who showed that the late cv. Greenfeast flowers later under a 16h photoperiod than under continuous light and those contained in the previous section on the critical photoperiod of L63.

MATERIALS AND METHODS

Suppression of *Sn* Activity by Continuous Light.

EXPERIMENT 1

This was a factorial experiment combining two photoperiods - continuous light (P24) and 18h (P18) with two varieties - lines 58 *(If e sn hr)* and 53 *(If e Sn hr)* and the four treatments C, D6, DO and E described below. Eighteen plants were used per factorial combination.

Treatment C-control. The seed was planted at the normal depth (2-3cm) and the cotyledons were never exposed. Shoots emerged on days 7 and 8.

Treatment D6. The seed was germinated under the same conditions as Treatment C but the cotyledons and shoot were exposed on day 6. At this time the radicles were 4-6cm long and the plumules 1-2 cm long.

Treatment DO. The intact seedlings were exposed to the appropriate photoperiod from the start of germination. The seeds were germinated in glass Petri dishes between two thin blankets of wet cotton wool. Sterile conditions were used with 10 seeds and 20 ml of water per dish. The seedlings were transplanted into the 3 litre cans (leaving the cotyledons exposed) on days 3 and 4 as their radicles attained a length of approximately 1 cm. All

testae were removed. The seedlings were watered frequently for the first few days and protected by a muslin screen.

Treatment E - embryos. The seed was germinated as for treatment DO and the cotyledons were excised as soon as the seed had imbibed sufficiently, which was 18-27h from the start of imbibition. The embryos were cultured on agar slopes (Whites Medium) and transferred to the 3 litre cans when they had expanded the leaf at node three.

The plants received about 13h of daylight, the remainder of the photoperiod being supplied by a mixed incandescent-fluorescent source giving an intensity of approximately 1600 lux at plant height. All treatments spent 18h per day in the main glasshouse chamber with the temperature ranging from 20-28°C. The P18 and P24 treatments entered separate compartments for the remaining 6h in which the temperature was held at 20°C. The results are contained in fig. 3.3.

EXPERIMENT 2

Seeds of lines 58, 53 and 63 *(lf e Sn Hr)* were germinated on a single layer of wet cotton wool in glass Petri dishes and planted out on day 5. They were exposed to 8h of light from a mixed fluorescent-incandescent source with an intensity of 20,000 lux at plant height, followed by 16h of light from incandescent bulbs with an intensity of 100 lux. The results are tabulated in table 3.1. The temperature was 17.5°C.

Determination of the Length of a Dark Period Required to Observe *Sn* Activity.

Lines 24 *(Lf e Sn hr)*, 53 and 58 were exposed to an 8h natural photoperiod and then moved to dark compartments where they were given supplementary light from a mixed incandescent-fluorescent source with an intensity of 1600 lux at plant height to complete the photoperiod of 18, 20 or 24h. The plants were germinated either on a layer of wet cotton wool in Petri dishes and then transplanted to the surface of the cans on days 4,5 and 6 (i.e. exposed to the photoperiod from the start of germination) or germinated 3 cm below the surface of the growing medium (i.e. cotyledons buried throughout the growth of the plants). The day temperatures varied between 14 and 33°C and the night temperatures between 15 and 21°C. The results are tabulated in table 3.3.

The Nature of the Substance Controlling the Photoperiod Response.

This experiment involved two lines, 58 and 53, with their cotyledons either shaded or exposed to the photoperiod of either 8 or 18h from day 5. The plants were either left intact, decotyledonised on day 5, had leaf 4 shaded from the time it was fully expanded (L4Sh) or were grafted in the combinations 58/53 and 58/58. The grafting was performed on day 5. Some treatments (58, intact and 53, L4Sh) did not have their cotyledons exposed to the photoperiod due to space considerations (see table 3.4). Shading in all treatments was done by wrapping the relevant organ in aluminium foil. This proved difficult in graft treatments since the rapid growth of cotyledonary laterals pushed the foil aside. Plants were therefore checked twice daily to ensure as complete shading as possible, any laterals being removed. Only vigorous grafts were used in the calculation of the results which are contained in table 3.4.

RESULTS

L53 *(lf e Sn hr)* plants are no later than L58 *(lf e sn hr)* plants when exposed to continuous light from the start of germination under the conditions used in experiments 1 and 2. (fig. 3.3,treatment P24DO; table 3.1). This indicates that the difference between genotypes *lf e sn hr* and *lf e Sn hr* can be completely eliminated by continuous light, regardless of whether the light is from a mixed fluorescent-incandescent source or from a weak,pure incandescent source. L63 *(lf e Sn Hr)* plants are also no later than L58 plants (or L53 plants) (table 3.1) indicating that the gene *Hr* does not significantly delay the flowering node when the expression of the gene *Sn* is suppressed by continuous light.

The above result for the comparison of lines 53 and 58 was observed consistently the first four times this experiment was performed and was subsequently reported (Murfet and Reid, 1974). However, further experiments using the same techniques showed inconsistencies in the behaviour of L53, its flowering node being up to 2.5 nodes later than L58 (significant at the 0.001 level). A typical set of results is shown in table 3.2. Considerable variation occurred amongst the L53 plants and it was decided to examine this variation to see whether it was heritable. Five seeds from each of the plants in the first sample of L53 used in table 3.2 were planted under continuous light. An analysis of variance between the various progenies showed that significant variation was occuring between the progenies at the 0.001 level. The regression of the mean flowering node of the progeny on the parental flowering node was $y = 0.21x + 8.32$ and was significant at the 0.05 level (fig. 3.4). Seed production by the progeny plants was poor but all available seed from 21 of

these plants (number of seeds/plant varied from 1 to 5) was grown under an 8h photoperiod. The regression of mean progeny flowering node on the parental flowering node was y = 1.15x + 10.38 and was significiant at the 0.01 level (fig. 3.5). These results suggest that L53 is heterogeneous for a polygenic system affecting the flowering node, the system being active in both continuous light and short days. Three of the families of L53 plants grown in continuous light flowered below the mean flowering node of a sample of 37 L58 plants grown at the same time. Therefore it is suggested that the reason for the inconsistencies in the behaviour of L53 grown in continuous light is this heterogeneity for the system of polygenic modifiers. It would appear that the earlier experiments used samples of L53 possessing an earlier polygenic background than did the later experiments. Presumably this heterogeneity arose due to the bulking of the line for physiological experiments before it was genetically pure. Once heterogeneity is present in a line the latest polygenic background is possibly selected, since the later a plant flowers the larger its yield is normally found to be. Over several generations this may lead to a build up of late modifiers in the line as seems to have occurred during the present study.

The previous statement that the genotypes *lf e sn hr* and *lf e Sn hr* flower at a similar early node if exposed to continuous light from the start of germination remains valid but in the light of the above results needs the added qualification - given an appropriate genetic background. Continuous light can clearly cause a marked reduction in the expression of gene *Sn* and the results are not inconsistent with a statement by Murfet and Reid (1974) that continuous light completely supresses the

activity of the gene *Sn*. However this statement cannot be made unequivocally until further experiments comparing either *Sn* and *sn* segregates in a segregating progeny or lines isogenic except for the alleles *Sn* and *sn* are performed since the crucial question in deciding whether light fully suppresses the expression of gene *Sn* is whether the genotypes *lf e sn hr* and *lf e Sn hr* would flower together given identical genetic backgrounds. How the quantitative system observed during this work operates is not revealed by the present results. Several possibilities exist and it will require a great deal of detailed work to eliminate the alternatives.

The length of the dark period required in a 24h cycle to allow an observable increase in *Sn* activity appears to be less than 4h since L53 plants exposed to a 20h photoperiod flowered significantly later than the plants exposed to continuous light (table 3.3) regardless of whether the cotyledons were exposed to the photoperiod or not (significant at the 0.01 level with the cotyledons buried and the 0.05 level with the cotyledons exposed). A similar result was obtained for L63 (fig. 3.2) but in L24 a significant delay was not observed until an 18h photoperiod was used (table 3.3). Whether this result with L24 reflects a function of the gene *Lf* or a difference in the polygenic background between the lines is uncertain, although the latter view is favoured. Under some circumstances the differences in the flowering nodes between plants grown under continuous light and 18 or 20h photoperiods were not significant (e.g. fig. 3.3, 53C and 53D6) and this probably reflects the slightly different temperatures used in the various experiments.

The data in table 3.3 also confirm several other res

reported either in this thesis or elsewhere. Firstly, L24 plants flower substantially later than L58 plants even under continuous light (5.4 nodes in this case) and this, at least partly, reflects the effect of gene *Lf* (Murfet and Reid, 1974). Secondly, L58 plants germinated in continuous light flowered 1.5 nodes later than plants germinated 3cm below the surface of the growing medium. This effect has been examined in detail in chapter 7.

In table 3.4 the short-day grafts 58/53 flowered over 4 nodes later than intact L58 plants. This delay cannot be attributed to either the absence of L58 cotyledons or the act of grafting itself (58/58) even though self-grafting has caused a significant delay. These results point strongly towards a positive delaying action by the L53 cotyledons *(lf e Sn hr)* which may be attributed to the formation of a graft-transmissible inhibitor by the *Sn* gene under SD. This delaying effect is lacking under the 18h photoperiod, supporting the earlier finding that the expression of the gene *Sn* is suppressed in long photoperiods. These results have been discussed in depth by Murfet and Reid (1973).

It is possible that the photoperiod response is caused by the differential production of a flowering stimulus in the shoot (as suggested by Amos, 1974) and that this difference is only observed in plants capable of inhibitor production. However under continous light (fig. 3.3) the difference between the flowering nodes for lines 53 and 58 falls from 5 nodes, where the cotyledons are buried in the usual manner, to 2 nodes where the cotyledons and plumule are exposed from day 6, to zero where the cotyledons and plumule have been exposed to light from the

start of germination. It is clear that the difference in flowering node is directly related to the period the cotyledons and plumule have spent in darkness. Since there is strong evidence in favour of the delay by stocks of genotype *lf e Sn hr* being caused by a flower inhibitor it seems most likely that the production of the inhibitor is under photoperiod control. This is supported by the fact that the 58/53 grafts exposed (including their cotyledons) to an 18h photoperiod flower significantly earlier (at the 0.01 level) than those grafts with their cotyledons kept in the dark, implying that the shoots cannot be responsible for the photoperiod effect. The 5 node difference between lines 53 and 58 when planted in the usual manner in LD therefore appears to be due to dark-formed inhibitor produced by the *Sn* gene in the cotyledons. Evidence suggesting that light renders *Sn* ineffective by suppressing *Sn* activity rather than by destroying the flower inhibitor produced by *Sn* is as follows. The flowering node of the intact L53 LD plants is only slightly less than the value of 14.94 which was obtained for the graft of 58/53 in which the scions were grown under short days and the cotyledons of the stock were kept in darkness. This indicates that continuous exposure to light has not led to the destruction of inhibitor in the L53 shoots, since the delay in each case represents the inhibitor contributed by one set of L53 cotyledons. It also follows that the inhibitor responsible for delaying *lf* shoots above node 15 (e.g. 53 SD) must be produced in the shoot itself.

If leaf 4 is kept in complete darkness while the rest of the shoot is exposed to an 18h photoperiod (table 3.4) the flowering node is delayed (significant at the 0.05 level). This result supports the suggestion that inhibitor is produced in the shoot as well as in the cotyledons (Murfet, 1973b) and illustrates

that the leaves have the ability to perceive the photoperiod. If the rest of the plant is exposed to SD, shading of leaf 4 causes a small but insignificant delay. This delay is probably not significant because initiation does not occur till much later (approximately 6 nodes) and secondly, because a large quantity of inhibitor would already be present. Exposure of the cotyledons in SD resulted in a small, but significant (at the 0.01 level) delay in the flowering node of L53 plants. This delay is not compatible with the previous interpretation of the results and it is suggested that it was due to the more vigorous growth of the plants with their cotyledons exposed. Vigorous growth has been shown previously to delay the flowering node of late cultivars under SD conditions (Reid and Murfet, 1974).

DISCUSSION

It is now clear that *Sn* is active in both the cotyledons and the shoot (leaves?) of genotype *lf e Sn hr* and that in both areas the activity is sensitive to light. Paton (1971) has also noted the competence of the cotyledons of the late cv. Greenfeast to function as foliage leaves. In Greenfeast sensitivity to photoperiod was not achieved before the 4th day with full competence developing between the 4th and 7th days. The present results with genotype *lf e Sn hr* suggest that the cotyledons are sensitive to light, at least on some genetic backgrounds, from the time *Sn* activity is possible. However, in the absence of treatments between day 0 and day 6 it is not possible to say when *Sn* activity commences. Paton (1969) is uncertain of a relationship between a flower inhibitor in peas and the response to photoperiod but the present evidence definitely indicates *Sn* as the cause of a photoperiod response and if the evidence that *Sn* forms a flower inhibitor (Murfet and Reid, 1973) is accepted,

we are drawn to the conclusion that inhibitor is directly concerned in the photoperiod response. The present work shows that photoperiod is regulating *Sn* activity rather than long days destroying inhibitor as suggested by Barber (1959). Further *Sn* activity appears to start soon after dark (at least less than 4h in lines 53 and 63) even though it may not be observed under some circumstances until longer dark periods are used since the promotor to inhibitor ratio may be well away from the threshold for flowering. The length of the preceding light period may affect the time after dark at which *Sn* activity begins. This problem along with questions on how the regulation of *Sn* activity is achieved have been examined in the following section and the results presented here only appear valid if fairly long light periods (e.g. 18 - 20h) are used.

The Effects of Light Quality and Quantity on the Flowering of Genotype *lf e Sn Hr*.

INTRODUCTION

The control of the photoperiod response in long day plants has been studied extensively (see reviews by Evans, 1971 and Vince, 1972). However, since the ability to respond to photoperiod is extremely variable, even within genera, as shown by the number of genera and even species which contain representatives of long day, day neutral and short day types (e.g. *Themeda australis;* Evans and Knox, 1969) it would seem unlikely that exactly the same mechanism would have evolved in the separate plant groups. Certainly the one pigment, phytochrome, appears to be implicated in all species, although even here the so-called high energy responses (Borthwick et al., 1968) have not been shown conclusively to have their action through this pigment. Variation in the responses to light breaks in the middle of the long night and to photoperiod extensions with blue light do occur within long day plants as do the wide array of responses to applications of the various growth regulating substances suggesting that differing mechanisms may occur. In general, light containing a mixture of red and far-red light is most effective in causing flowering of long day plants, the most effective ratio depending on the length of the exposure and time during the cycle at which it is given (Vince, 1972). Both Vince and Evans (1971) explain this by suggesting that there are reactions requiring both high and low amounts of Pfr. Schneider et al. (1967) suggest that two photoreactions occur, one through phytochrome and one which has an action spectrum between 710-720 nm and which Borthwick et al. (1968) suggest may be caused by the photo-dissociation of a Pfr - substrate complex. This second reaction falls into the class known as high energy reactions (HER).

Pisum contains representatives of both long day and day neutral types of plants. In the late pea cultivar GW, which is a quantitative long day plant, Nakamura (1965) showed that a 1h light break from a mixed fluorescent - incandescent source in the middle of a 16h night, caused a promotion of the flowering node, illustrating that a true photoperiod response occurs in peas. Marx (1969) found that a 6h extension of the 9h main photoperiod of natural light with "TL" No. 32 fluorescent tubes did not result in a promotion of the flowering node to the same extent in his G-type cultivar, 1326, as did natural 15h days or a photoperiod extension with mercury vapour lamps plus incandescent bulbs. He suggested that the differing response arose because of the different light qualities involved. The present study was designed to find which wavelengths and intensities of light were most effective in promoting the flowering node of peas when used as either night breaks or photoperiod extensions. The genotype *lf e Sn Hr* was used exclusively through the work since it has previously been shown to be very sensitive to light over a relatively long period. It was hoped that since the gene *Sn* confers the ability to respond to photoperiod on peas this work would reveal further information on the control of the activity of the gene *Sn* by light. The results were also analysed to show whether a relationship exists between the control of flowering in peas and endogenous rhythms as suggested by Bünning (1936).

MATERIALS AND METHODS

Seeds were planted 2 to 3cm below the surface of the growth medium, L63 *(lf e Sn Hr)* being used in all experiments. The plants in experiments 1 to 8, with the exception of those given continuous white light, received an 8h photoperiod of

natural light and were then moved to dark compartments where they received various combinations of darkness and light from sources with known intensity and spectral properties (see tables 3.5 and 3.6). The plants given continuous white light received natural light extended to 24h with light from a mixed fluorescent-incandescent source, giving an intensity of approximately 1600 lux at plant height. Twenty-four plants were planted in all treatments, with the exception of the continuous light and 16h dark treatments. The number of scoreable plants in each treatment is indicated in the relevant table of results. Impenetrant plants were excluded from the results in all experiments since they behaved as day neutral plants (with respect to the flowering node). For a discussion of the characteristics of these plants see Murfet (1971a, 1973a,b).

Red light was obtained by filtering light from Mazda white fluorescent tubes through one 3mm layer of red 400 perspex, and had an intensity of 20 $\mu W/cm^2$ at the top of the growth medium unless otherwise stated. Far-red light was obtained by filtering light from a 100 W pearl incandescent bulb through a 10cm layer of water, 3mm of glass and 3mm of FRF 700 plexiglas and had an intensity of 180 $\mu W/cm^2$. Blue light was obtained by filtering light from Mazda white fluorescent tubes through a 3mm layer of blue perspex and had an intensity of 16 $\mu W/cm^2$. The amount of red and far-red light in this source was below the level of detection by our techniques. Light sources with different spectral outputs were obtained by using Mazda pearl incandescent bulbs, Philips PF712 bulbs, Sylvania gro-lux tubes and Mazda white fluorescent tubes. The Philips PF712 bulbs and the gro-lux tubes have similar red to far-red ratios to the incandescent bulbs and white fluorescent tubes respectively but have

increased proportions of their output above 600nm. The light intensities for these sources with differing spectral compositions were measured using a Hewlett-Packard radiant flux meter and detector which gives a flat response over the wavelength range of 300 to 3000nm. The percentage of red to red plus far-red light in the sources was determined by measuring the amount of light tramsmitted by Schott AL band filters of wavelengths 657nm & 728nm. The ratio of the intensity at 657nm to the intensity at 728nm plus 657nm for the various sources are given in table 3.5. It should be noted that these results were found to vary by at least 2% depending on the age of the tubes or bulbs. Where only mixed incandescent-fluroescent sources were used, the light intensities were measured using a simple light meter which measures the output only in the visible wavelengths. This seemed more reliable in these circumstances due to the high output of infra-red radiation in the locality of the incandescent bulbs and the consequent difficulty in obtaining a true reading with the flux meter.

In all experiments, except numbers 3, 6 and 7 in table 3.6 and numbers 9, 10 and 11 in table 3.9, the plants were exposed to the various combinations of light treatments indicated in tables 3.6, 3.7, 3.8, 3.9 and 3.10 and fig. 3.6, from the time the plumules broke the surface of the growing medium. In experiments 3, 6 and 7 the plants were grown in SD conditions for 20 days before transfer to the appropriate conditions (the number of leaves expanded being 6 to 7), while in experiments 9, 10 and 11 the times of transfer to the experimental conditions are indicated in table 3.9. All plants were then exposed to the appropriate conditions until the age (from start of germination) indicated in the appropriate table of results. After this time the plants, with

the exception of those given continuous light in table 3.8, were either transferred to the general glasshouse if flower buds were observable in all plants of a particular treatment, or returned to an 8h photoperiod for between 20 and 30 days and then to the apron to mature. By this latter treatment it was possible to determine whether or not the treatment had, in fact, caused floral initiation. In treatments where between 0 and 100% flowering was observed the mean flowering nodes are of little value in the analysis of results since they are computed on the total scoreable plants. Where relevant the mean flowering node of the plants induced by the treatment is given in the text.

In the experiment using the 16h extensions with fluorescent and gro-lux tubes (table 3.8) the plants received one LD cycle (32h of light) before transfer to SD at the completion of the experimental treatment. (At this stage twelve leaves were expanded and by dissection the total number of nodes was 23.50± .29). This probably induced any plants that had not already flowered in these two treatments.

Plants given cycle lengths other than 24h or involving a 4h main photoperiod (tables 3.9 and 3.10) received only artificial light from a source consisting of 6 x 40 W cool white Mazda fluorescent tubes and 4 x 40 W pearl incandescent bulbs with a total intensity of approximately 23,500 lux at plant height. Details of the cycle lengths are contained in the relevant table of results. Due to the length of some cycles some treatments in experiment 10 were completed at slightly different times (see table 3.9). Where no treatments in an experiment flowered under the experimental conditions, the experiment was concluded by exposing plants to several LD

cycles. It was hoped that this process would allow the separation of treatments possessing sub-threshold promotor to inhibitor ratios (see table 3.10). In all these experiments (tables 3.9 and 3.10) the temperature was 17.5°C.

RESULTS

From table 3.6 (experiment 1) it can be seen that a 16h extension of an 8h photoperiod with far-red light causes flowering about 10 nodes earlier than a similar treatment with red light. Red light is promotory when compared with blue light, which had no effect, the plants not flowering until after transfer to LD conditions. When a 2h light break was given in the middle of a 16h night, far-red light had no effect (table 3.6, experiment 2). Plants given a 2h red light break flowered 27 nodes earlier than the SD controls, but were still 9 nodes later than plants given continuous white light. Plants given a night break of red light (in experiment 2) flowered 4 nodes earlier than plants given a 16h extension with red light (in experiment 1). These results would suggest at least two actions of light in controlling the flowering node of peas, one in which far-red light is most active and long exposures are required and a second, in which red light is active and short durations only are required.

When 1h of red light was given for the hour before the middle of a 16h night, flowering was again promoted (table 3.6, experiment 3). Two hours of far-red light given in the 2h after the middle of the night resulted in 6 plants being induced and 18 not being induced before transfer back to SD for development. After transferal to LD conditions for maturing, the other 18 plants flowered, resulting in a bimodal distribution of the flowering

nodes for this treatment, the mean for all 24 plants being shown in table 3.6 (experiment 3) along with the percentage caused to flower by the treatment. The six early plants had a mean flowering node of 32.00 ± 0.69 while the other 18 plants had a mean flowering node 55.89 ± 0.79, which is not significantly different from the SD control plants. When 1h of red light was followed by 2h of far-red light the flowering node was delayed by 3.65 nodes, when compared to the plants given only 1h of red light (significant at the 0.001 level). These plants did not flower at a significantly different node to the six plants that flowered under 2h of far-red light. It therefore appears that the reaction to a night break of red light is partially reversible by far-red light. This would suggest that phytochrome is involved in at least one of the reactions which controls the response to photoperiod in peas. The reason six plants flowered in response to 2h of far-red light is possibly because the plants are becoming so sensitive to light by the time 32 nodes have been laid down (see p.76), that 2h of far-red light each night results in flowering via the reaction in which far-red light is most active. It could not be working through the reaction in which red light is active since far-red light is inhibitory to this action of red light.

If red light is given for the second 8h of a 16h night period 100% flowering is caused with a mean flowering node of 21.1 (table 3.6, experiment 4). This flowering node is significantly lower (at the 0.001 level) than that observed in plants given either continuous red light or a 1 or 2h red light break in the middle of a 16h night period. However, caution needs to be used in the interpretation of these differences since the results came from separate experiments. If 8h of far-red light is given during the first 8h of a 16h night 30% flowering results.

Eight hours of far-red light followed by 8h of red light resulted in 100% flowering, with a mean 1.1 nodes earlier than those plants given 8h dark followed by 8h red light (significant at the 0.001 level). Eight hours red light followed by 8h dark resulted in 11% of plants being induced (experiment 5). Therefore, even though the results came from 2 separate experiments, it appears that 8h red light in the second half of the night is highly promotory while 8h in the first half of the night is only slightly promotory. Eight hours far-red light followed by 8h dark caused only 53% of plants to flower, the flowering node of these 9 plants being 33.78 ± 1.13, while 8h dark followed by 8h far-red light caused 100% of plants to flower, with a mean of $25.71 \pm .67$ (experiment 6). If the 8h of far-red light was given in the middle of the 16h night, 100% of plants were induced to flower at a mean of $29.24 \pm .79$. It therefore appears that 8h of far-red light is more promotory the later in a 16h night that it is given. Plants given 8h of red light followed by 8h far-red light behaved similarly to plants given 8h red light and 8h dark (experiment 5), and had a reduced percentage of flowering when compared to plants given 8h dark plus 8h of far-red light (significant at the 0.001 level). This may not be a true null effect of the far-red light in the 8h red, 8h far-red treatment, since the far-red light broke down on 4 nights out of the total of 30 days treatment. This result needs further clarification.

The minimum length of the red light break required in the middle of the night to induce flowering is less than fifteen minutes (table 3.6, experiment 7). The intensity of red light required is very low, $1\mu W/cm^2$ being sufficient to induce 100% flowering when given as a 2h night break (table 3.7). However, there is an interaction between the intensity required and the

duration of the break (table 3.6, experiment 7) suggesting that a certain quantity of light needs to be received before the break is effective. Although 1μW/cm^2 caused 100% flowering, the flowering node observed was significantly higher than in the 5μW/cm^2 treatment (significant at the 0.001 level). The three higher intensities did not give significantly different results (table 3.7).

The intensity of light required to be effective as a 16h photoperiod extension was shown to be less than 60μW/cm^2 of incandescent light. However, a significant decrease in the flowering node (at the 0.001 level) was observed as the intensity was increased (table 3.7). Philips PF 712 bulbs, incandescent bulbs and white fluorescent and gro-lux tubes were all effective in causing flowering, whether given as a 16h photoperiod extension after 8h of natural light or as 2h light breaks in the middle of a 16h night (table 3.8). However, they were not equally promotory, incandescent bulbs being significantly (at the 0.001 level) more promotory than Philips PF712 bulbs which were more promotory (at the 0.001 level) than fluorescent or gro-lux tubes under both sets of conditions. The fluorescent and gro-lux tubes did not yield significantly different results whether used as photoperiod extensions or night breaks, but the results for the photoperiod extension experiment are suspect for these two sources due to the plants accidentally receiving one LD cycle (see Materials and Methods). The difference between the results for Philips PF712 and incandescent bubls, when given as photoperiod extensions, could have been due to an intensity difference, although this seems unlikely since the intensities used were substantial in both cases. Comparison of the intensities between the bulbs and tubes is invalid due to the high output of

infra-red radiation by the former. However, the intensities used appear to be almost saturating.

The length of the dark period required before a light break given during a 16h dark period is effective, appears to be between 4 and 5h (fig. 3.6). If continued growth under the conditions was possible a smaller length of time may be required due to the increased sensitivity of L63 plants to light as they age (chapter 3). However, 5h darkness before the light break is not as effective as 6 or 8h, since, although all three treatments caused 100% flowering, the flowering node was significantly lower (at the 0.001 level) for the 6 and 8h treatments. Possibly of more significance is the fact that a period of over 12h is required after the start of a light period before a light break is effective in promoting flowering. This occurs whether the main photoperiod is of 8h (fig. 3.6) or 4h (table 3.9, experiment 11) duration and suggests that the start of a photoperiod in some way makes the plant unresponsive to further light breaks for a certain period. However, the size of the promotion by the light break is reduced when the photoperiod is reduced from 8 to 4h (significant at the 0.001 level), suggesting that the extra light is still capable of an observable promotory effect once the light break becomes effective.

In experiment 8 (table 3.6) the length of the dark period required before a night break can be effective was examined by exposing plants to an 8h photoperiod of white light followed by either 0, 2, 4 or 6h of red light before being exposed to 2h of red light from the fourteenth hour after the start of the main photoperiod. The results suggest that a dark period of greater than 2h is required before the night break can

be effective, since the 2R 4D 2R 8D treatment is significantly more promotory (at the 0.001 level) than either the 8R 8D or 4R 2D 2R 8D treatments. The last two treatments are not significantly different. The reason that the 6D 2R 8D treatment is significantly earlier than the 2R 4D 2R 8D treatment (at the 0.001 level) is not clear, although it is possible that 4h of darkness is just on the threshold of being long enough for a night break to be effective and that this varies slightly as the plant ages.

Varying the length of the dark period from 12 to 60h whilst retaining a constant 12h photoperiod, did not show any rhythmic variation in the flowering behaviour of L63 (table 3.9, experiment 10) as has been shown to occur in the LD plant *Hyocyamus niger* by Hsu and Hamner (1967). With dark periods of 24 to 60h no flowering was induced in any of the plants. In the 12h dark treatment 81% of the plants were induced by the treatment, indicating that even a 12h photoperiod can cause induction of L63 plants, provided it is given at an age where the plants are sensitive enough to respond. A 12h dark period would be expected to be one of the most inhibitory conditions if a rhythm was occurring. If the length of both the light and the dark period are varied (from 12 light: 12 dark to 24:24 and 36:36) a substantially different result is observed (table 3.9, experiment 9). Exposure of plants to a 12h light and 12h dark period produced 15% flowering whereas both the 24 and 36h treatments produced 100% flowering. This result would not indicate any firm evidence for an endogenous rhythm occurring in peas since the 24 and 36h treatments had almost identical effects. It would however, suggest that either less inhibitor is produced during 24 and 36h of continuous darkness, than in 2 or 3 twelve hour periods of darkness respectively, (consistent with what

would be expected if a rhythm was occuring) or else that during 24h and 36h of continuous light the ratio of the flowering hormones can be shifted sufficiently to allow induction to occur before the next period of darkness commences. The author favours the latter view since it has been shown that L63 can be induced by one LD cycle (32h of light) (page 76). The difference between the results for the 12h light: 12h dark treatments in experiments 9 and 10 was due to the longer duration of the treatment and the older age of plants at its completion in experiment 10.

The results in table 3.10 show that plants receiving different sub-threshold conditions for flowering can be separated by exposing them to a number of LD cycles and recording the percentage of plants induced in each treatment. This test system is therefore suitable for comparing promotor to inhibitor ratios in plants where this would normally be hidden if the plants were grown continuously under the experimental conditions. Further, the results in table 3.10 show no rhythm in the sensitivity of L63 plants to a 2h light interruption of a 38h dark period since the 10L 12D 2L 24D and 10L 24D 2L 12D treatments are not significantly different. If the light break is given after 30h darkness (10L 30D 2L 6D) it is less promotory than after 12h darkness (not significant) and 24h darkness (significant at the 0.05 level), but more promotory than if it is given in conjunction with the main 10h photoperiod (treatment 12L 36D)(significant at the 0.01 level). Therefore, although the results display no rhythm as has been observed in *Hyoscyamus niger* (Hsu and Hamner, 1967) and *Sinapis alba* (Kinet et al., 1973) they do show that a light interruption of a long dark period does increase the promotor to inhibitor ratio when compared to plants given the

extra light in conjunction with the main photoperiod. This result is similar to those found when a 16h dark period is interrupted by a night break and is thought to act by the same mechanism.

DISCUSSION

The results indicate that 2 reactions are involved in the control of the photoperiod response in L63. The first reaction requires long durations of light and is optimally expressed when the light source possesses a high proportion of far-red light, although red light does have a small effect. A possible explanation of the reaction is that a complex of some type is formed prior to inhibitor production. If this complex is broken down on illumination with wavelengths between 700 and 730nm continuous illumination with wavelengths in this region would result in the inhibition of inhibitor production. Red light and fluorescent light appear slightly effective in this reaction and this could be caused by the small amount of energy within the critical range of wavelengths that are produced by these sources. On page 40 it has been shown that less than 4h of darkness in a 24h photocycle will result in an inhibition of the flowering node in plants of genotype *If e Sn Hr*. This would support the above explanation, since the time for which inhibitor production is stopped by the start of the photoperiod would have elapsed (see second reaction) and inhibitor production would therefore start as soon as the breaking down of the complex by the far-red wavelengths had ceased.

The second reaction is sensitive to red light and only requires a short night break (as little as 15 minutes) to cause a large promotion of the flowering node. The night break is only

effective if given at least 13h after the start of the previous photoperiod and at least the last 2h of this period must be in darkness. The effect of a 1h night break of red light can be partially reversed by far-red light. These results suggest that this reaction is controlled by phytochrome and that the ratio of Pfr/Pr needs to be raised above a certain threshold for flowering to occur. For this switch type mechanism to operate a period of darkness prior to exposure to red light is required for the Pfr/Pr ratio to fall to a level below the threshold. The present results suggest that a dark period of between 2 and 4h is required and this agrees well with the results of Furuya and Hillman (1964) who showed by spectrophotometric means in vivo that about 80% of the initial Pfr formed by exposure of pea seedlings to red light had disappeared after 2h darkness and over 90% after 4h darkness. All types of lights used in the present experiments, except the far-red light, allow the Pfr/Pr ratio to increase above the threshold and consequently cause flowering. It would appear that the ratio of Pfr/Pr needed to allow flowering is therefore between the level formed by far-red light and the Philips PF712 bulb. The ratio of Pfr/Ptotal formed by a far-red source of the type used is about 1% and for the Philips bulb is probably 50-60% if the results of Borthwick et al. (1969) are assumed to be applicable. Unfortunately it was not possible during the course of the present study to obtain light sources giving stable Pfr/Ptotal ratios between these levels. After the threshold of Pfr/Pr required for flowering is passed it appears that inhibitor production (controlled by the *Sn* gene) cannot commence for a certain period of time (or at least cannot proceed at the previous rate), since only a short period of red light is required to cause a large promotion of the flowering node. The length of this period is substantially shorter than the 13h required after

the start of a photoperiod before a night break is effective, since both Barber (1959) and Haupt (1969) have shown an 8h photoperiod to be more inhibitory than a 12h photoperiod in L types of peas. The results in fig. 3.6 show that a 2h break in a 16h night is more effective (although not statistically significant) when given after 8h darkness than after 10h. This may suggest that inhibitor production is turned off for over 4h after the completion of the night break, or possibly of more importance, for over 6h from the start of the night break. The results in fig. 3.6 suggest the maximum period that inhibitor production would be stopped could be little more than 8h from the start of the light break, although a more accurate technique would be required to specify the length of the period precisely. The reason why night breaks after 5 and 6h darkness are not as effective as those after 8h is not clear. It might be expected that night breaks with red light after 5 and 6h darkness would be as effective as those after 8h darkness, since once sufficient time had elapsed from the start of the main photoperiod for the plants to respond to night breaks they would cause the same effect (provided the next main photoperiod did not start until the night break had had its full effect). Similarly, it might be expected that 8h of far-red light would have a similar effect, regardless of when it was given in a 16h night, if it is assumed that the start of the main photoperiod prevents inhibitor production for a period of less than 8h. In neither case was this observed, the light being more effective when given later in the 16h night. A possible explanation is that although inhibitor production is possible between 6 and 8h after the start of the photoperiod this ability does not reach a maximum until some time later. Whether it reaches a plateau or is rhythmic in its behaviour is not indicated by these results.

The results which show that an 8h photoperiod is more promotory than a 4h photoperiod (table 3.9, experiment 11), provided a night break is given so that the difference can be observed, may be used to suggest that light stops inhibitor production for a period of somewhat less than 8h. However, one should be cautious when interpreting these results, since an alternative explanation of the results is that the red night break is acting as the photoperiod which prevents any further response to a light break for 13h. Thus, the white light of the "main" 8 or 4h photoperiod would only be acting via the first reaction in which far-red light is most effective and in which the size of the response is thought to be directly related to the length of the exposure. Only further experiments will show which is the true explanation of these results.

Since the results for Philips PF712 bulbs and incandescent bulbs and for fluorescent tubes and gro-lux tubes are so similar, it appears that the ratio of red to far-red light far outweighs the quantity of light at wavelengths below 600nm or the ratio of light greater than 600nm to that below 600nm. The result with a blue extension of the main photoperiod would support this conclusion.

The effect of various light types on the induction of flowering in peas is fairly typical, if such a thing exists, of the responses shown by other LD plants. However, unlike *Hyocyamus niger* (Schneider et al., 1967), an extension of a short photoperiod with blue light is ineffective. Another plant with similar responses is *Lolium temulentum*, except that a light break in the middle of the long night is not effective in inducing *L. temulentum* (Evans, 1969). These two examples are sufficient

to show that although there are distinct similarities in the photoperiod responses of LD species there are also distinct differences. The proposed mechanism for the photoperiod response has definite similarities to that proposed by Schneider et al. (1967), especially in that 2 light dependent reactions are proposed in both cases. The reaction which appears to involve phytochrome because of its red/far-red reversibility suggests that high amounts of Pfr promote flowering in LD plants at certain times. This is similar to suggestions by Parker et al. (1950), Evans (1972) and Vince (1972). The other reaction, which is most promotive when the plants are given protracted irradiation with wavelengths above 700nm is not as clear cut. The explanation put forward is similar to the suggestion put forward by Schneider et al. (1967) and elaborated by Borthwick et al. (1969) to suggest that the photo-dissociation of a Pfr-substrate complex is involved. Evans (1971) and Vince (1972) suggest that far-red light is active because low Pfr processes are also involved, low Pfr levels being promotive soon after the high intensity period. In peas, both red and far-red light are more effective if given in the second half of a 16h night. Also, continuous light containing both red and far-red light is the most promotory condition, and if the view put forward by Evans (1971) and Vince (1972) is accepted, this implies that both the low and high Pfr processes can occur at the one Pfr level. This level would be relatively low in peas, since far-red light is almost as promotory as white light, even though its intensity is considerably less (table 3.6, experiment 1). For these reasons the type of mechanism proposed for the first reaction by Borthwick et al. (1969) has been favoured in the interpretation of the present data.

It has not been possible to show the involvement of

endogenous rhythms in the control of flowering in peas by the use of experiments with different cycle lengths or light perturbations of long dark periods, even though threshold conditions were achieved (tables 3.9 and 3.10). This could possibly be due to the inability of the experimental design to show their presence (e.g. incorrect light intensities, or the rhythms possess a cycle substantially different from 24h or the rhythm damps out after one cycle in continuous darkness, as in *Xanthium* (see Salisbury, 1969)) or to their non-existence. There are, however, large changes in the sensitivity of the plant to both red and far-red light which are often used to suggest the occurrence of rhythms. This explanation is inconclusive since, even in an hour-glass model similar to the one suggested this variation would not be unexpected. The effect of interruptions of the night with red light could be interpreted as phase shifts of a rhythm, the red light acting as a dawn signal, as has been suggested to occur in *Xanthium* (Salisbury, 1969). Whether this is the case cannot be determined until further experiments are carried out. However, even if a rhythm is present in peas, the results so far can be most simply interpreted in terms of an hour-glass mechanism, this mechanism being sufficient to fully interpret the flowering behaviour of peas exposed to natural photoperiods.

The two reactions controlling the photoperiod response in the genotype *If e Sn Hr* presumably act by controlling the amount of inhibitor produced by the *Sn* gene, since this gene has been shown to confer the photoperiod response on peas (Barber, 1959; Murfet, 1971a). However, nil effects of some light treatments should not be interpreted as indicating that the activity of the *Sn* gene has not been altered, since, under some conditions the promotor to inhibitor ratio may be well

away from the threshold for flowering and consequently quite large changes in the amount of inhibitor produced may not result in an altered flowering node.

Under natural conditions the photoperiod extremes met by peas are probably 8 to 20h. The second reaction would therefore play relatively little part in controlling the photoperiod response in peas unless the length of time between the start of the photoperiod and the time that inhibitor production could start was considerably greater than 8h and this does not appear to be the case. I suggest that in peas the major timing factor for the photoperiod response is the amount of time per day that the *Sn* gene is active and that this activity is determined by the photoperiod via the first reaction. This would be supported by the fact that many factors which can effect the activity of the *Sn* gene, and consequently the promotor to inhibitor ratio (e.g. age, temperature and genotype (Murfet, 1971a; Murfet and Reid, 1974; chapters 4 and 5)) also effect the photoperiod response. This means that there is no need to postulate a temperature and age independent time measuring system in the control of the photoperiod response in peas.

Table 3.1 Mean node of first initiated flower ±S.E. for lines 58 *(lf e sn hr)*, 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)* given 8h of white light and 16h of weak incandescent light each day from the start of germination.

L58		L53		L63	
$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
13.52 ± .37	19	13.30 ± .36	20	12.26 ± .37	19

Table 3.2 Mean node of first initiated flower ± S.E. for lines 58 *(lf e sn hr)* and 53 *(lf e Sn hr)* exposed to continuous light from the start of germination. Three separate batches of L53 seed were used.

L58		L53(1)		L53(2)		L53(3)	
$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
10.76 ± .16	17	12.28 ± .37	18	13.11 ± .32	18	12.78 ± .27	18

Table 3.3 The mean node of first initiated flower ±S.E. for lines 58 (*lf e sn hr*), 53 (*lf e Sn hr*) and 24 (*Lf e Sn hr*) with their cotyledons either exposed to the photoperiod from the start of germination (D0) or buried 3 cm below the surface of the growing medium (D∞). The plants were exposed to a photoperiod of either 18, 20 or 24h light.

Photoperiod	58,D0	53,D0	58,D∞	53,D∞	24,D0
24	12.00 ± .47	12.63 ± .46	10.53 ± .13	14.57 ± .17	17.38 ± .27
20	11.63 ± .44	14.19 ± .37	10.27 ± .12	15.47 ± .19	17.93 ± .23
18	12.27 ± .41	15.00 ± .35	10.19 ± .10	15.53 ± .22	19.61 ± .14

Table 3.4 Mean node of first initiated flower ±S.E. for lines 58 *(lf e sn hr)* and 53*(lf e Sn hr)* with their cotyledons either shaded or exposed to either an 8 or 18h photoperiod from day 5. The plants were either left intact, decotyledonised (-), grafted on day 5 (e.g. 58/53) or had leaf 4 shaded (L4Sh).

Photoperiod	18 hour				8 hour			
Cotyledons	Shaded		Exposed		Shaded		Exposed	
	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n
53,intact	14.29 ± .16	21	12.57 ± .24	21	21.00 ± .49	15	23.47 ± .70	15
58,intact	10.22 ± .22	23			10.17 ± .12	24		
58/53	12.47 ± .17	17	11.67 ± .16	15	14.94 ± .17	18	14.47 ± .19	15
58/58	11.47 ± .13	15	11.90 ± .23	10	10.89 ± .16	18	11.33 ± .17	9
53,-	12.27 ± .18	15			17.50 ± .49	14		
58,-	10.58 ± .15	24			10.38 ± .25	24		
53,L4Sh.	14.90 ± .14	20			22.28 ± .55	14		

Table 3.5 The percentage of red light to red plus far-red light in the light sources used during the present study.

SOURCE	% $\frac{\text{Red}}{\text{far-red + red}}$
RED	89.7
FAR-RED	$<$.02
FLUORESCENT TUBE	89.3
GRO-LUX TUBE	97.3
INCANDESCENT BULB	44.4
PHILIPS PF712 BULB	43.8
BLUE	Undetectable

EXPERIMENT	TREATMENT	% FLOWERING	FLOWERING NODE ($\bar{X}$±S.E.)	n	AGE (days)
1	16L	100	14.52±.25	21	56
1	16R	100	28.21±.50	19	56
1	16FR	100	18.18±.54	17	56
1	16B	0	42.89±.40	19	56
2	16L	100	14.83±.51	12	61
2	7D 2R 7D	100	23.63±.35	19	61
2	7D 2FR 7D	0	45.33±.88	18	61
2	16D	0	48.00±.96	15	61
3	16L	100	18.83±.17	12	52
3	7D 1R 8D	100	27.95±.46	19	52
3	8D 2FR 6D	25	49.92±2.24*	24	52
3	7D 1R 2FR 6D	100	31.60±.29	20	52
3	16D	0	57.13±2.83	8	52
4	16L	100	17.25±.13	4	41
4	8D 8R	100	21.10±.24	21	41
4	8FR 8D	29	41.00±2.00*	21	41
4	8FR 8R	100	19.95±.15	21	41
4	16D	0	44.83±1.02	12	41
5	16L	100	14.75±.41	8	45
5	8R 8D	11	45.95±1.52*	19	45
5	8D 8FR	58	33.67±2.17*	21	45
5	8R 8FR	11	45.37±1.43*	19	45
5	16D	0	52.83±1.25	6	45
6	16D	0	51.38±1.96	13	64
6	8FR 8D	53	39.41±1.69*	17	64
6	8D 8FR	100	25.71±.67	14	64
6	4D 8FR 4D	100	29.24±.79	17	64
7	16L	100	19.14±.34	7	63
7	7.75D .5R 7.75D	100	31.87±.32	23	63
7	7.75D .25R 8D	27	52.50±2.19*	22	63
7	7.75D .25RH 8D	100	32.75±.59	20	63
7	16D	0	63.18±1.08	11	63
8	8R 8D	85	38.70±2.30*	20	60
8	4R 2D 2R 8D	73	41.64±2.80*	22	60
8	2R 4D 2R 8D	100	31.67±.37	9	60
8	6D 2R 8D	100	26.63±.37	19	60
8	16D	0	64.22±2.96	9	60
8	16L	100	14.91±.16	11	60

* This flowering node is the mean for all plants given a particular treatment.

Table 3.7 The mean node of first initiated flower ±SE. for L63 plants exposed to an 8h photoperiod and then transferred each day to dark compartments and given either 16h of light from incandescent bulbs of 4 different intensities or 7.5h of darkness, 1h of red light at 4 different intensities followed by a further 7.5h of darkness till day 60.

INTENSITY ($\mu W/cm^2$) of INCANDESCENT	CONTINUOUS $\bar{x}$ ± S.E.	n	INTENSITY ($\mu W/cm^2$)	1 HOUR FLASH $\bar{x}$ ± S.E.	n
8,200	14.08±.25	24	56	25.55±.41	20
1,800	14.87±.17	23	20	25.28±.32	18
200	15.36±.36	22	5	24.47±.24	15
60	15.25±.36	24	1	27.67±.54	18

Table 3.8 The mean node of first initiated flower ± SE. for L63 plants exposed to an 8h photoperiod followed by either 16h of light or 7h darkness, 2h light, 7h darkness. The light was provided by either Philips PF712 bulbs, incandescent bulbs, fluorescent tubes or gro-lux tubes at the intensity specified. The age of the plants at the completion of treatment was 52 days.

LIGHT TYPE	INTENSITY	CONTINUOUS		2 HOUR FLASH	
		$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
RUBY-RED	800 $\mu W/cm^2$*	17.33±.19	21	23.33±.20	21
INCANDESCENT	800 $\mu W/cm^2$	15.65±.13	20	21.81±.29	16
FLUORESCENT	95 $\mu W/cm^2$	23.18±.20	17	24.48±.20	21
GRO-LUX	80 $\mu W/cm^2$	23.29±.19	17	25.16±.31	19

* In the continuous treatment the intensity was only 300 $\mu W/cm^2$.

Table 3.9 The percentage of plants induced to flower by the experimental treatment and the mean node of first initiated flower ± S.E. for L63 plants exposed to the varying cycles of white light (L) and darkness (D) indicated in the treatment column. The duration of each treatment is indicated in hours preceding the treatment symbol. The light was from a mixed incandescent-fluorescent source. The number of plants scored (n) and the age at the start and finish of the treatment is indicated.

Experiment	Treatment	Percent flowering	Flowering node $\bar{x}$ ± S.E.	n	Age (days) Start	Finish
9	12L 12D	15	45.31 ± 2.01	13	46	63
9	24L 24D	100	26.80 ± .49	5	46	63
9	36L 36D	100	26.88 ± .35	8	46	63
9	8L 16D	0	46.38 ± .78	8	46	63
10	12L 12D	81	41.50 ± 1.77	20	50	71
10	12L 24D	0	57.72 ± .99	18	50	71
10	12L 36D	0	53.77 ± 1.88	13	50	70
10	12L 48D	0	55.84 ± 1.24	19	50	70
10	12L 60D	0	51.11 ± 1.82	9	50	71
11	4L 6D 2L 12D	0	57.89 ± .87	19	29	45
11	4L 8D 2L 10D	0	59.29 ± 1.18	17	29	45
11	4L 10D 2L 8D	100	34.60 ± 1.05	20	29	45
11	8L 6D 2L 8D	100	28.00 ± .22	20	29	45

Table 3.10 The percentage of L63 plants induced to flower by either 1,2,3 or 4 LD cycles (first cycle 32h, then multiples of 24h) after exposure to cycles of varying lengths (indicated in hours) of light (L) and dark (D) from the time the shoot emerged until day 29. The light was from a mixed incandescent-fluorescent source. After exposure to the LD cycles the plants were transferred to SD conditions on the trucks.

TREATMENT	Number of LD Cycles							
	1		2		3		4	
	% flowering	n	% flowering	n	% flowering	n	%flowering	n
12L 36D	0	25	0	24	19	26	44	25
10L 12D 2L 24D	0	24	16	25	46	26	88	25
10L 24D 2L 12D	0	28	33	27	66	29	89	27
10L 30D 2L 6D	0	24	11	27	41	27	69	29

Fig. 3.1 The effect of age on the number of LD cycles (first cycle 32h of light, then multiples of 24h) required to induce 50 per cent (solid lines) and 100 per cent (broken lines) flowering in lines 53 *(lf e Sn hr)*, 63 *(lf e Sn Hr)* and 24 *(Lf e Sn hr)*. The points have been interpolated from the raw data of two separate experiments. The plants were grown in an 8h photoperiod on the trucks before and after treatment.

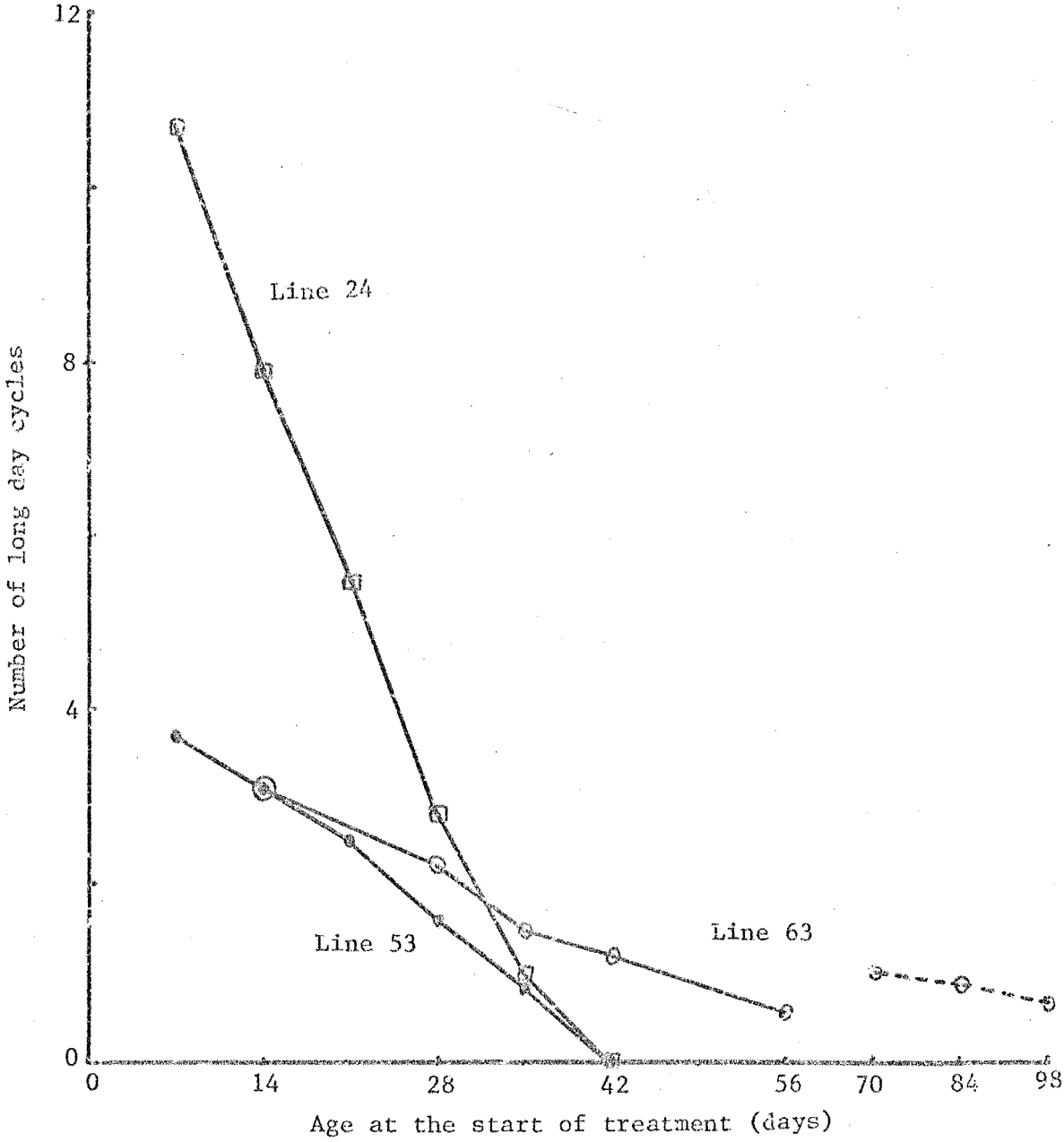

12
8
4
0
Number of long day cycles
Line 24
Line 53
Line 63
0
14
28
42
56
70
84
98
Age at the start of treatment (days)

Fig. 3.2 Mean node of first flower $\pm$S.E. for L63 plants exposed to 12, 13, 14, 15, 16, 18, 20 and 24h photoperiods from a mixed incandescent-fluorescent source at a temperature of 17.5°C. Under the 13h photoperiod only 8 plants (36%) were induced before the completion of treatment when 25.74$\pm$.23 leaves were expanded. The point for 13h on the graph comes only from these 8 plants. No plants were induced to flower in the present experiment by a 12h photoperiod. The minimum number of plants scored per treatment was 16.

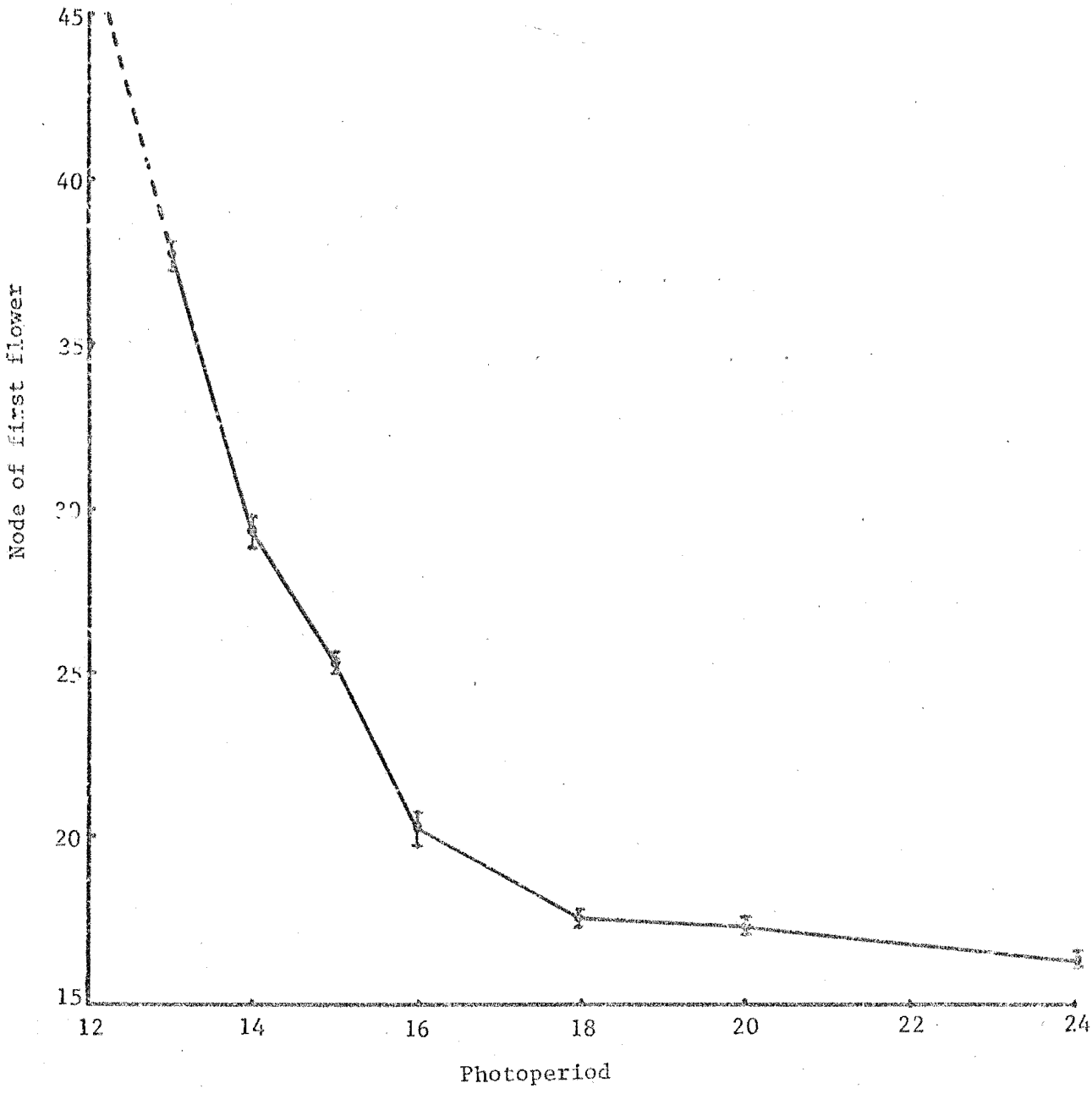

45
40
35
30
25
20
15
Node of first flower
12
14
16
18
20
22
24
Photoperiod

Fig. 3.3 Mean node of first initiated flower ± S.E. for plants of L53 *(lf e Sn hr)* and L58 *(lf e sn hr)* grown in a photoperiod of 18h (P18) or continuous light (P24) and given the following treatments: seed planted (buried) in the usual manner (C); the cotyledons and shoot exposed from day 6 (D6) or from the start of germination (D0); embryos excised from the cotyledons 18-27h from the start of imbibition, the appropriate photoperiod applying from the start (E). Eighteen plants were used per treatment.

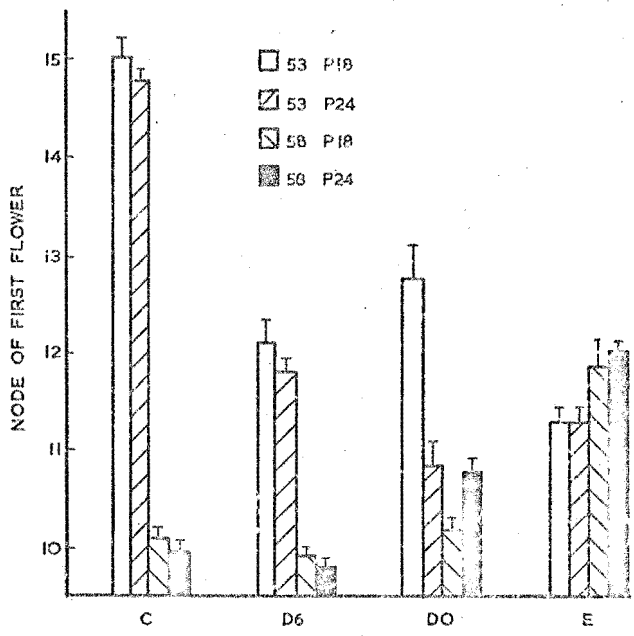

NODE OF FIRST FLOWER
15
14
13
12
11
10
53 P18
53 P24
58 P18
58 P24
C
D6
DO
E

Fig. 3.4 Regression of mean flowering node for the progeny of L53 plants *(lf e Sn hr)* plotted against the flowering node of the parent ($y = 0.21x + 8.32$). All plants were exposed to continuous light from the start of germination. The slope of the regression is significantly different from 0 (at the 0.05 level).

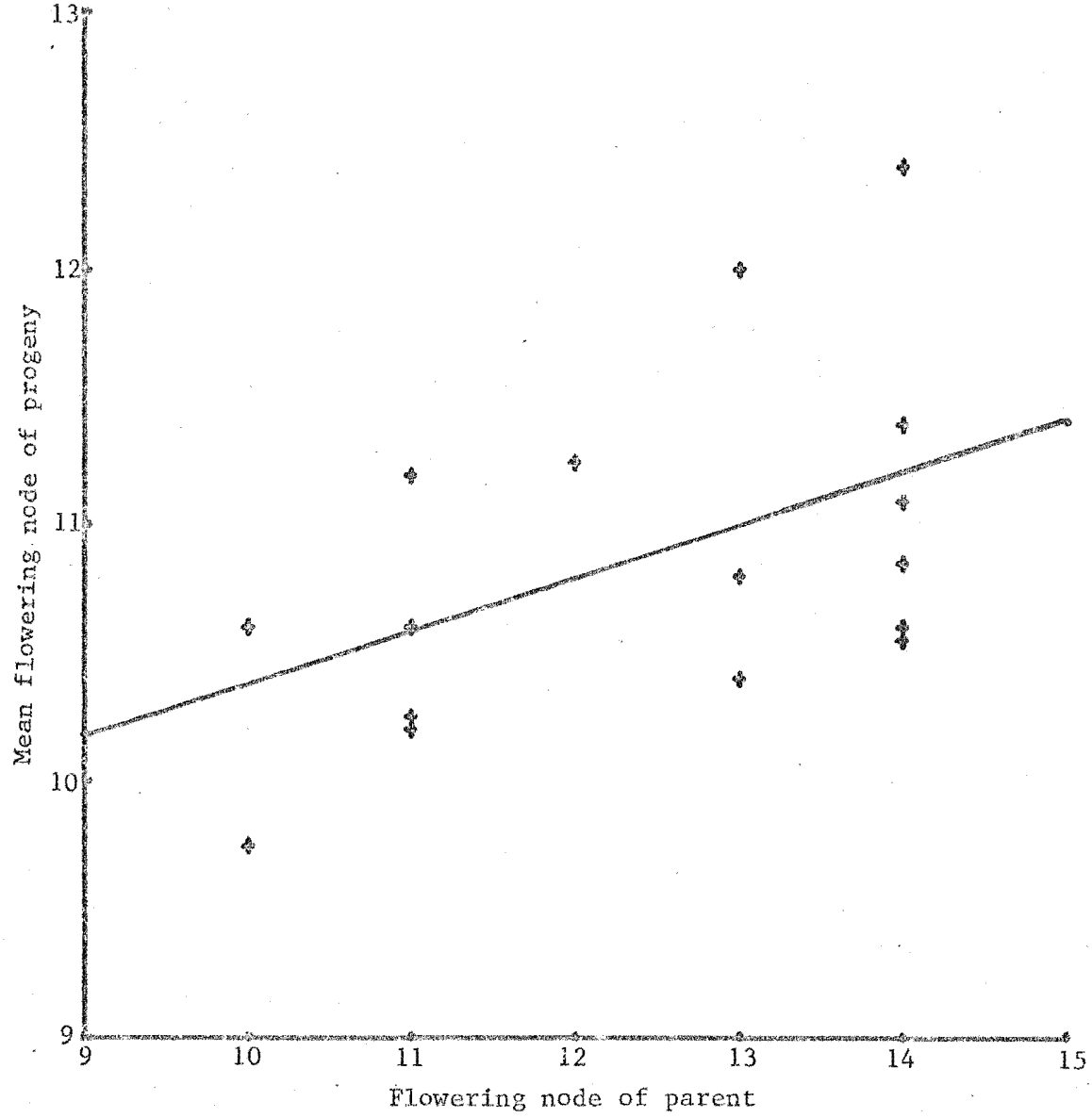

Mean flowering node of progeny
13
12
11
10
9
9
10
11
12
13
14
15
Flowering node of parent

Fig. 3.5 Regression of mean flowering node of progeny of L53 plants *(lf e Sn hr)* plotted against the flowering node of the parent ($y = 1.15x + 10.38$). The parental plants were exposed to continuous light from the start of germination while the progenies were exposed to an 8h photoperiod. The slope of the regression is significantly different from 0 (at the 0.01 level).

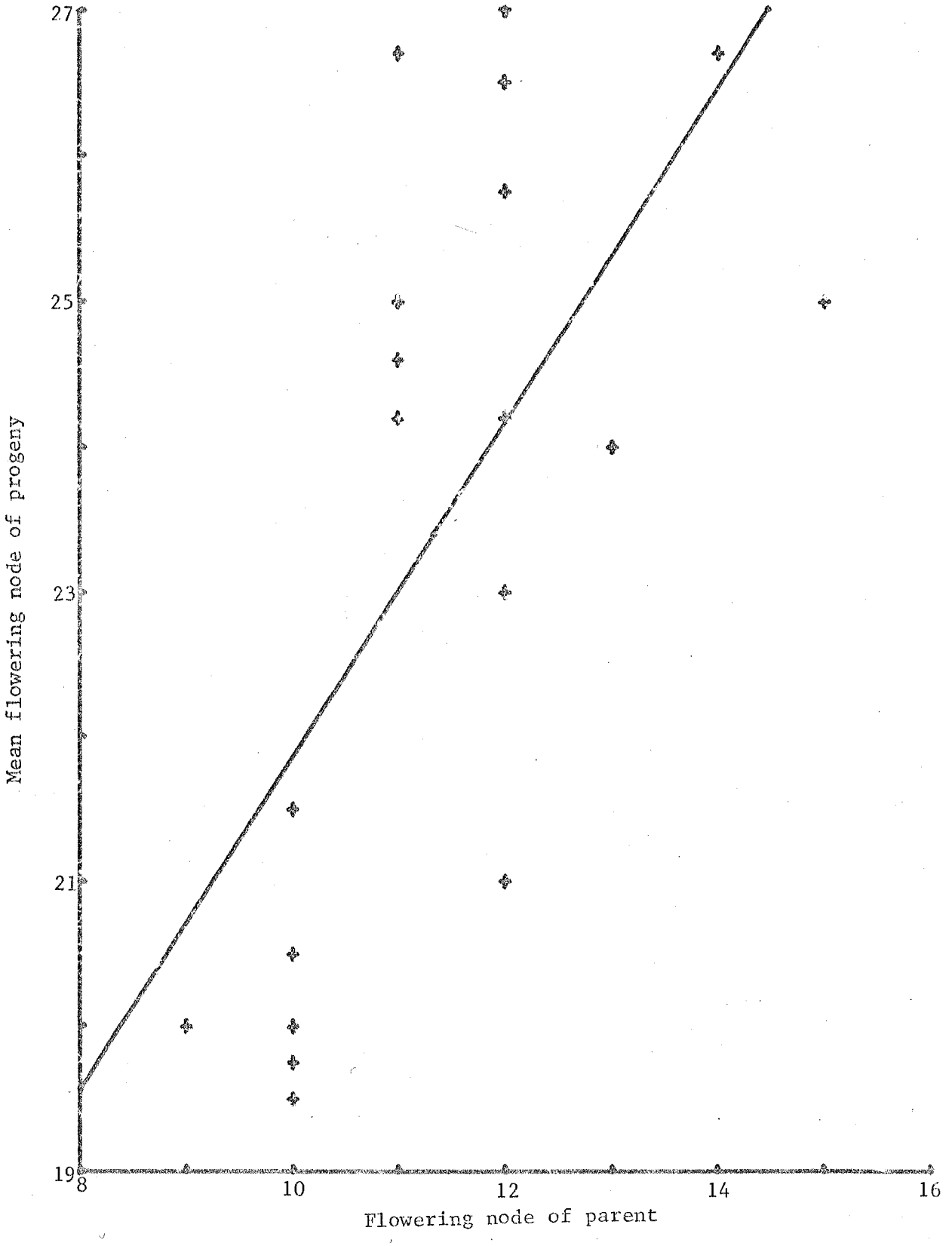
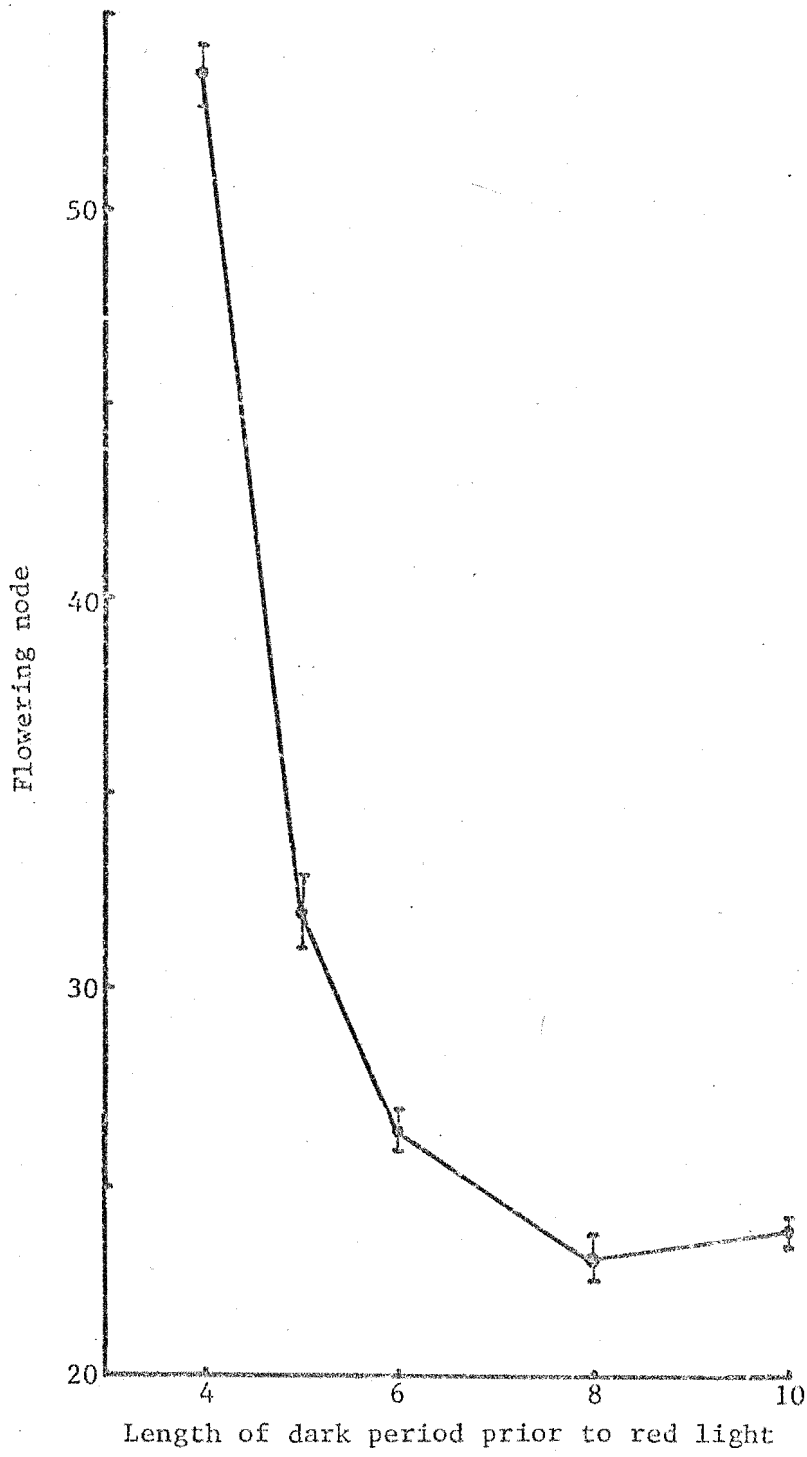

27
25
23
21
19
Mean flowering node of progeny
8
10
12
14
16
Flowering node of parent

Fig. 3.6 The mean node of first initiated $\pm$ S.E. for L63 plants *(lf e Sn Hr)* exposed to an 8h photoperiod followed by a 16h night interrupted by 2h of red light after either 4,5,6,8 or 10h darkness. The plants exposed to 4h darkness prior to treatment with red light did not initiate until after transfer to a long photoperiod.


Flowering node
50
40
30
20
4
6
8
10
Length of dark period prior to red light

CHAPTER 4

TEMPERATURE

The Sites and Possible Mechanisms of the Vernalisation Response.

INTRODUCTION

Vernalisation generally reduces the flowering node of late cultivars of peas but early cultivars show either no response to vernalisation or a slight negative response (Barber, 1959; Haupt, 1969; Murfet and Reid, 1974). Paton (1969) showed by grafting experiments that vernalisation eliminates the ability of stocks of the late cv. Greenfeast to increase the flowering node of scions of the early cv. Massey and he suggested that low temperatures repress synthesis of a flower inhibitor in the cotyledons of Greenfeast. He also found that the interval between the completion of photoperiodic induction and the evocation of flowering at the apex was longer in unvernalised plants as opposed to vernalised plants and he attributed this delay to the presence of the flower inhibitor in unvernalised plants. Similarly, Amos and Crowden (1969) have proposed that vernalisation has two effects in Greenfeast, one in the shoot where it predisposes young plants to the photoinductive processes and a second smaller effect through a decrease in the amount of cotyledonary inhibitor. Neither Paton nor Amos and Crowden envisage any clear connection between photoperiod and the level of flower inhibitor. However, Barber (1959) reported that a single dominant gene, *Sn* confers, not only a high flowering node, but also an ability to respond to both photoperiod and vernalisation and that long days and

vernalisation act competitively to reduce the flowering node. His observations have been supported by our own results (Murfet and Reid, 1974). For example, if genotype *lf e Sn hr* is exposed to continuous light from the start of germination no vernalisation response is observed. Likewise, if vernalisation is followed by continued cold (2-4^{o}C) night temperatures no photoperiod response is observed. However, in chapter 3 it has been shown that light causes the repression of *Sn* activity, rather than the destruction of the inhibitor as suggested by Barber, and a similar mechanism is favoured for the effect of cold temperatures (Murfet and Reid, 1974).

In the present study the techniques of grafting and cotyledon removal were used to identify the site(s) at which vernalisation has an effect in several different genotypes. From these results an endeavour has been made to draw some inferences on the mechanisms involved in the response. Genotype *lf e Sn hr* was included since it shows the vernalisation and photoperiod responses typical of a late cultivar and genotype *lf e Sn Hr* since preliminary studies had shown both the photoperiod and vernalisation responses to be enhanced by this gene combination. An early flowering line of genotype *lf e sn hr* was also included. This genotype normally shows no response to vernalisation but it was considered that the graft procedure may reveal some effect of vernalisation otherwise covered up in intact plants. A vernalisation response is also known to occur in genotype *Lf e sn hr* but has not been investigated in the present study.

MATERIALS AND METHODS

All treatments received an 8h photoperiod of natural light supplemented when necessary with light from a mixed incandescent, fluorescent source. Grafting and cotyledon removal were performed as described in chapter 2. The unvernalised plants were planted five days before it was thought the vernalised plants would be ready, grafting being performed as soon as the vernalised plants were removed from the cold room. From the time of grafting all cotyledons were exposed to the photoperiod.

Experiment I

This experiment was designed to differentiate between the effects of vernalisation on the cotyledons and on the plumule of L 63 *(If e Sn Hr)*. The experiment consisted of 8 treatments: unvernalised intact plants (UV), vernalised intact plants (V), unvernalised decotyledonised plants (UV-), vernalised decotyledonised plants (V-) and the four grafts UV/UV(unvernalised scion and stock), V/V, V/UV and UV/V. The vernalised plants received temperatures of between 2 and 4°C for the first 35 days. After three weeks the grafts were scored for number of leaves expanded and separated into vigorous or non-vigorous grafts; grafts having less leaves expanded than the decotyledonised plants were considered non-vigorous. Twenty-four plants were used per treatment and night temperatures were between 14 and 20°C and day temperatures between 20 and 30°C. The plants were transferred to long days when approximately 31 leaves had expanded.

Experiment II

This experiment was likewise designed to identify the site(s) of the vernalisation response but in addition we sought to establish whether a positive response to vernalisation could

occur in the absence of the dominant genes *Lf* and *Sn*. The experiment consisted of 17 treatments: UV, V, UV- and V- for lines 58 (*lf e sn hr*) and 53 (*lf e Sn hr*) and grafts 53UV/53V, 53UV/53UV, 53V/53UV, 53V/53V, 58UV/53UV, 58V/53UV, 58UV/53V, 58UV/58UV and 58UV/58V. The three remaining graft combinations, 53V/58V, 58V/58UV and 58V/58V were not performed due to time and space limitations. The experiment was carried out twice, 30 plants per treatment being used each time. The grafts 53UV/53UV, 53V/53UV, 53UV/53V, 58UV/53UV and 58V/53UV were repeated for a third time because of the low success rates of grafts which had a vernalised section. Only vigorous grafts were used in the calculation of results in fig. 4.1, this being decided from the length between nodes 1 and 6 and from the number of leaves expanded about three weeks after grafting. The length of vernalisation in the three trials varied but the plants were the same size when vernalisation was completed. The number of days of vernalisation was 46, 39 and 33 respectively, the temperature being between 2 and 4^{o}C. The results from the three trials were homogeneous except that the 58V and 58UV- treatments were delayed with respect to the 58UV treatment in the first trial but not in the second. The night temperatures were between 14.7^{o}C and 21^{o}C and the day temperatures between 14^{o} and 36^{o}C. The significance of the difference between treatment means was determined by the use of Students t test. Most treatments gave unimodal data but in the case of the graft 53UV/53V the flowering node values were distributed into a group of 9 plants flowering at node 15 or lower and a group of 3 plants flowering at node 19 or higher. In each test involving this graft the significance level was found to be the same whether these three plants were included or excluded.

RESULTS

The data in table 4.1 confirm the previous evidence (Murfet, 1973a; chapter 3) that unvernalised plants of L63 flower at a very high node under short days (in this case the mean flowering node is 39.8). Vernalisation caused a substantial decrease in the flowering node, the distribution breaking up into two distinct groups with mean flowering nodes of 12.8 and 21.5. This discontinuity does not represent genetic heterogeneity but arises from the threshold nature of the flowering process and the failure of the hormonal balance to surpass the critical level in some plants. The resulting bimodality is very similar to that which occurs in L61a at normal growing temperatures. The underlying circumstances in L61a are discussed by Murfet(1973b).Overall the flowering node of L63 plants can be separated into three regions in table 4.1; a low region (nodes 10-16), a middle region (nodes 17-24) and a high region (nodes 29-49)

The promotion of flowering to the low region represents the largest vernalisation response reported in peas and appears to result from some effect of vernalisation on the cotyledons since two thirds of the vigorous UV/V grafts but none of the V/UV or V- plants flowered in this region. Flowering in the middle region appears to result from some effect of vernalisation on the scion (embryonic leaves or apex) since all V- plants and V/UV plants but no UV/V plants flowered in this region. Flowering in the high region required that the scion be unvernalised since all UV/UV grafts and one third of the UV/V grafts flowered in this region. It is interesting to note that all the slow grafts (in brackets in table 4.1) flowered in the middle or high regions and therefore acted as decotyledonised plants as suggested by Murfet (1971c).

Vernalisation promoted flowering by 9 nodes in intact L53 plants (fig. 4.1) which agrees with previous results (Murfet and Reid, 1974). The 53UV/53UV and 53V/53V grafts are not significantly different from the respective intact controls illustrating that the act of grafting itself had little or no effect in vigorous grafts. The 53V/53UV graft flowered 3 nodes earlier than the 53UV/53UV graft (significant at the 0.01 level) while the 53UV/53V graft flowered 5.5 nodes earlier than the 53UV/53UV graft (significant at the 0.001 level) but was still significantly (at the 0.01 level) later than the 53V/53V graft. The V- plants flowered 3.5 nodes earlier than the UV- plants (significant at the 0.001 level) which is similar to the 3 node difference between the 53V/53UV and 53UV/53UV grafts. These results indicate that vernalisation has an effect both on the stock and on the scion in plants of genotype *If e Sn hr*. In contrast to the results of Amos and Crowden (1969) for Greenfeast, the effect in the stock is larger than the effect in the scion for lines 53 and 63.

Cotyledon removal caused a four node promotion of flowering in unvernalised plants of L53 (significant at the 0.001 level) and a one node delay in vernalised plants (significant at the 0.01 level). The former effect is by now well known and is discussed by Murfet (1976) and in chapter 5. In the UV, UV- and V- plants flowering is probably dependent on the phasing-out of *Sn* activity in the shoot but with vernalised intact plants the flowering node is low enough to fall within the sphere of influence of the cotyledons which appear to have actively stimulated evocation.

Vernalisation had a small (0.27 node) but significant (0.01 level) delaying effect on intact plants of L58. This

agrees with previously reported data for intact early developing varieties (Barber, 1959; Haupt, 1969). Vernalisation of the decotyledonised plants of L 58 caused a 0.65 node decrease in the flowering node (significant at the 0.001 level). However, it is not clear if this is a direct effect on flowering or the result of altered vegetative growth since the vernalised plants had longer stems between nodes 1 and 6 (significant at the 0.001 level). In unvernalised plants of L 58 both cotyledon removal and self grafting led to small increases in the flowering node but again the higher flowering node was associated with a reduction in the vigour of the plants. The 58UV/58UV grafts were not significantly different from either the vernalised intact plants or the 58UV/58V grafts. Whatever the explanation of the small effects described above, these results provide no evidence to suggest that vernalisation of the cotyledons of genotype *lf e sn hr* leads to a promotion of the flowering node.

Several effects are evident from the 58/53 grafts. The 58UV/53UV grafts were 4 nodes later than the 58UV/58UV grafts which supports the previously reported hypothesis that cotyledons of genotype *lf e Sn hr* produce a flower inhibitor under short days (Murfet and Reid, 1973). Vernalisation of the stock (58UV/53V) resulted in a 2.5 node promotion (significant at the 0.001 level) but the plants were still 1.5 nodes later (significant at the 0.01 level) than the 58UV/58UV control. In contrast Paton (1969) reported that vernalisation completely eliminated the ability of stocks of cv. Greenfeast to delay flowering in scions of cv. Massey. Vernalisation of the 58 scion resulted in a 0.8 node promotion (difference 58UV/53UV-58V/53UV significant at the 0.01 level). The latter result was checked to see if altered vegetative growth could have been responsible.

Neither the length of the stem between nodes one and six nor the number of leaves expanded after 4 weeks were significantly different between the two treatments suggesting that the effect was a direct one on the flowering process. Therefore, scions of genotype *lf e sn hr* can show a small positive response to vernalisation when grafted to *lf e Sn hr* stocks.

DISCUSSION

It is clear that there are at least two sites of vernalisation in the late lines. This is particularly evident in the case of L 63 which, because of its potentially large response to such environmental factors as temperature and photoperiod, has proved an excellent experimental line. Vernalisation of L 63 *(lf e Sn Hr)* stocks (cotyledons) produced a 26 node promotion and vernalisation of the shoot a 19 node promotion compared with 5.5 and 3 nodes respectively in L 53 *(lf e Sn hr)*. However, the underlying physical or chemical changes are not necessarily greater in L 63 as elaborated below.

The vernalisation effect in the shoot is not dependent on the presence of genes *Sn* and *Hr* in the shoot since scions of L 58 *(lf e sn hr)* are also vernalisable but the gene combinations *Sn hr* and *Sn Hr* certainly magnify the effect as illustrated by the graft sequence 58UV/53UV-58V/53UV, 53UV/53UV-53V/53UV, 63UV/63UV-63V/63UV where promotion of the flowering node is 1, 3 and 19 nodes respectively. The present experiments do not identify the mechanism involved in the scion effect. Paton's (1969) transfer studies suggest an effect at the apex. If vernalisation lowers the threshold ratio of promotor to inhibitor required for flowering the same reduction in threshold could readily lead to responses of quite different magnitude in the graft sequence above as a result of differences in the rate of

change of the hormone levels. Inhibitor levels would be expected to fall rapidly in L58 scions with the demise of the cotyledons on the L53 stocks. In the 53/53 grafts inhibitor levels will be maintained for a somewhat longer time as a result of *Sn* activity in the shoot. In the 63/63 grafts the presence of *Hr* may prolong *Sn* activity for an extended period leading to a slow change in hormone levels. This line of reasoning also raises the possibility of a second mechanism contributing to the scion effect, especially in *Sn* shoots. Vernalisation alters the relationship between chronological and plastochronic age and the aging mechanism may lead to a decline in *Sn* activity (or formation of a hormonal balance favouring flowering) after fewer nodes have been formed.

The reason why intact plants of L58 *(lf e sn hr)* show no positive response to vernalisation, when the shoots have the potential, is not clear but promotion of the flowering node below a mean of about 9.8 in this line may be precluded by the existence of a juvenile phase. On the other hand the effect of a lower apical threshold in the vernalised shoots may be offset by a less favourable balance emerging after vernalisation of the recessive *sn* cotyledons. Our experimental procedures are not sensitive enough to resolve the issue. However, with *Sn* stocks, although vernalisation has probably altered the levels of both promotor and inhibitor the net result is a balance more favourable to flowering and we suggest this would come about if the reactions leading to the formation of the inhibitor and promotor possess different temperature co-efficients, the inhibitor formative sequence having the higher co-efficient (Wareing and Phillips, 1970; Murfet and Reid, 1974). The low temperature repression of *Sn* activity is only observed in the

cotyledons in the present experiments since they comprise the bulk of the tissue during seed vernalisation but the same type of effect may also occur in the shoot if cold temperatures were given at a later stage of development since *Sn* is operative in both the shoot and cotyledons. The fact that a discontinuous bimodal distribution of the flowering node arises in the 63UV/63V grafts illustrates that if flowering does not occur soon after transfer to post-vernalisation conditions the ratio of promotor to inhibitor again decreases and flowering does not occur until either the aging process or transfer to long days again alters the hormonal balance in favour of flowering. These results, together with the previous data (Murfet and Reid, 1974), strengthen the evidence that long days and vernalisation both promote flowering, at least in part, by repressing *Sn* activity and the production of inhibitor. This conclusion agrees with Paton (1969) and Amos and Crowden (1969) regarding the effect of vernalisation in the stock but differs in respect to a connection between photoperiod and inhibitor which these workers consider uncertain or non-existent.

There are two established theories for the action of vernalisation (Lang, 1965; Purvis, 1966); firstly, that vernalisation affects the leaves and results in a hormonal balance in favour of flowering (Melchers, 1936, 1937) and secondly, that it "thermo-induces" cells, this state being transmitted only by cell division (Schwabe, 1954). This second effect is observed largely at the apex although a similar type of response has been observed in the leaves of *Lunaria biennis* by Wellensiek (1962). The present results suggest that peas possess both types of mechanism which, as suggested by Evans (1971), is not unexpected. The effect of low growing temperatures (5 to 15^{o}C)

on the two sites of vernalisation described in this section would be of interest since reports (e.g. Murfet, 1973a; McWilliam and Jewiss, 1973) have indicated that these intermediate temperatures can cause flowering in some plants under normally non-inductive photoperiod conditions.

Kinetics of the Vernalisation Responses in the Genotype *If e Sn Hr.*

INTRODUCTION

In the previous section it has been determined that two distinct sites of vernalisation occur in peas and that at least two, and possibly three, different mechanisms exist. The results presented here concern work which set out to determine the kinetics of the vernalisation responses with the aim of finding out whether they are consistent with the mechanisms proposed for these responses. It was also of interest to compare the kinetics for L63 to those reported by previous workers using L types of peas (Barber, 1959; Paton, 1969; Amos and Crowden, 1969) and other species (Schwabe, 1959; Lang, 1965; Purvis, 1966). The kinetics specifically studied included the time required for both the cotyledon and shoot effect of vernalisation to be manifest at 3°C, the range of temperatures which are effective in eliciting these responses and the effect of plant age and post-vernalisation temperatures on these responses.

MATERIALS AND METHODS

L63 *(If e Sn Hr)* plants were used throughout the present experiments since the response to vernalisation in this line is the largest yet reported for peas.

Length of Vernalisation Required in Intact Plants.

Plants were exposed to 0, 1, 2, 3 or 4 weeks vernalisation from the start of germination in the cold room at 2-4°C. Upon the completion of vernalisation the plants were exposed to an 8h photoperiod on the trucks until they were approximately three months old, after which time they were placed on the apron to

mature. Planting was done in early spring. The results are contained in table 4.2.

Length of Vernalisation Required to Elicit a Shoot Response.

Plants were exposed to 0,1,2,3,4 or 5 weeks vernalisation at 2-4°C from either the start of germination or from the time leaf 5 was fully expanded (24 days). The planting was staggered so that at the start of post vernalisation conditions all plants were at the same developmental stage. In order to eliminate the cotyledon effect of vernalisation the cotyledons were removed from all plants when the plumules were approximately 2cm long. After the completion of vernalisation plants were grown under an 8h photoperiod until 19 weeks old and then transferred to the apron. Planting commenced at the end of September. The analysed results are contained in table 4.3 while the raw data are contained in Appendix 1.

Time at which Vernalisation is Effective.

The plants received either no vernalisation or 4 weeks vernalisation at 2-4°C from either the start of germination (day 0), day 5, day 9 or day 13. Thirty two plants were grown per treatment. Planting was carried out in early December, plants being exposed to an 8h photoperiod on the trucks for 3 months and then to a long photoperiod on the apron until they were scored. The results are combined in table 4.4.

Temperature Required to Elicit a Vernalisation Response.

Plants were exposed to an 8h photoperiod at either 3,6,9,12°C or normal phytotron temperatures (average of 17°C) either from day 0 until day 38 or from day 0 until the plumule was between 2.5 to 3cm long (i.e. either for the same chronological

time or till the same physiological age was obtained). After this time all plants were exposed to an 8h photoperiod on the trucks until approximately 19 weeks old (approximately 38 leaves expanded) at which time the plants were transferred to the apron until ready for scoring. Planting commenced in mid-June, 30 plants being grown per treatment. The results are contained in table 4.5.

Effect of Post-Vernalisation Temperature.

Plants were given either 4 weeks of vernalisation at between 2 and 4°C or 3 days of growth in the phytotron before being exposed for two weeks to an 8h photoperiod of weak fluorescent light (3,200 lux) at 10,15,20,25 or 31°C. At the completion of this treatment all plants were grown under an 8h photoperiod in the phytotron until 35-40 leaves were expanded and then transferred to the apron. Planting of vernalised plants occurred in mid-November and of unvernalised plants in mid-December. All treatments except the 31°C contained 30 plants, the 31°C treatments containing only 24 plants. The results are contained in table 4.6 and Appendix I.

Stabilisation of the Devernalisation Response.

Plants were given 33 days of vernalisation at between 2 and 4°C before being transferred to an 8h photoperiod on the phytotron trucks. The latter treatment was either not interrupted or interrupted, after 0,1 or 2 weeks, by 2 weeks at 32°C. The photoperiod during this 2 week period was 8h from a mixed fluorescent-incandescent source with an intensity of 23,500 lux at plant height. Planting was done in August, 30 plants being grown in each treatment. The results are contained in table 4.7 (experiment 1).

Effect of Light Intensity on the Devernalisation Response.

All plants were given 32 days of vernalisation at between 2 and 4°C and then either put on the trucks under an 8h photoperiod or given 9 days at 30°C. The plants exposed to 30°C were given either complete darkness or an 8h photoperiod of either weak fluorescent light (3,200 lux) or high intensity light from a mixed incandescent-fluorescent source (32,000 lux). At the completion of this treatment all plants were placed on the SD trucks. The results are contained in table 4.7 (experiment 2).

Effect of Vernalising Temperatures on Developing Seed.

A group of 3 plants was exposed to an 8h photoperiod at 7.5°C from the time of anthesis until senescence occurred. The progeny from these plants and the progeny from a group of plants grown on the apron (average temperature 20°C) from the same batch of seed were planted under an 8h photoperiod on the trucks. A temperature of 7.5°C was used as the vernalising temperature since the temperature usually used (2-4°C) was not sufficient for seed development. The results are contained in table 4.8.

RESULTS

The cotyledon effect of vernalisation (i.e. the promotion of the flowering node of L63 to the low region by vernalisation) gradually becomes more pronounced as the length of vernalisation is increased from 1 to 4 weeks (table 4.2). However, even after 4 weeks vernalisation only 37% of plants were induced to flower in the low region (nodes 10-16). This low percentage is probably due to the high post-vernalisation temperatures experienced in this experiment (see discussion). No attempt was made to divide the plants into middle and high regions since these classes

ran together because the plants were transferred to the natural spring photoperiod at an early age and the planting was not staggered.

A significant shoot effect of vernalisation (i.e. the promotion of the flowering node of L63 to the middle region by vernalisation) is first observed after 3 weeks vernalisation when the vernalisation is given from the start of germination or after 2 weeks when the vernalisation is given after 24 days growth (table 4.3). The difference between these two results is similar to the length of time required for complete imbibition of seeds at 2-4^{o}C and consequently may not reflect a direct change in the sensitivity of plants to vernalising temperatures but just an inability to respond until the seeds are fully imbibed. These results (table 4.3) are based on a cut-off between the middle and high regions being placed at node 40 (node 40 is a zero point). However, similar results would have been obtained if the cut-off was placed anywhere between nodes 38 and 47 since only a few plants flowered at these nodes (see Appendix 1). Most of the plants close to the cut-off point received 1,2 or 3 weeks vernalisation from the start of germination and appear to have flowered at these nodes because of the vernalisation treatment and not because their growth rates were reduced. This indicates the quantitative nature of the shoot effect of vernalisation. This is further illustrated by the quantitative effect of vernalisation on the flowering nodes in the middle region — the longer the vernalisation the earlier the flowering node (significant at the 0.001 level for plants given vernalisation from day 0).

Regardless of the age at the commencement of vernalisation (at least between days 0&13) virtually all plants are shifted from the high region by 4 weeks vernalisation. However the results presented in table 4.4 indicate that the time at which the vernalisation is given has a marked effect on the distribution of the flowering nodes between the low and middle regions. If the vernalisation is given after 0 or 5 days 44% and 42% of plants respectively fit into the low region, but if given after 9 or 13 days this percentage drops to between 3 and 4% (significantly different from the 0 and 5 day treatments at the 0.001 level, $X_1^2 = 11.52$ between the closest 2 results). Prior to placement in the vernalisation chamber on day 9 the third leaf was fully expanded while by day 13 the fourth leaf was expanded. Although no dissections were carried out during this experiment it would appear that no more than 12 nodes would have been present on day 9 and 14 on day 13 if it is assumed that dissections recorded in chapter 2 are relevant. Consequently ample room is available for initiation in the low region provided the levels of the flowering hormome could be altered rapidly enough and to a sufficient degree by vernalisation. This does not occur to a large extent since, by the time treatment commences on days 9 and 13, the young shoot has become a major source of hormone production and, as will be shown later, the ratio of hormones produced by the young shoot is more inhibitory than that produced by the cotyledons. Consequently even though the vernalising temperatures increase the ratio of promotor to inhibitor they do not cause a sufficient increase to allow flowering. The lower ratio of promotor to inhibitor produced by the first foliage leaves when compared to the cotyledons appears to be the cause of the bimodal distributions of the flowering nodes under several sets of conditions in L & LHR

types of peas. It is interesting to note that the mean flowering node of the plants flowering in the low region and given vernalisation from day 0 is significantly lower (at the 0.001 level) than for those plants given vernalisation from day 5. This presumably results from a lower ratio of promotor to inhibitor being established over the first 5 days in plants not receiving vernalisation, flowering not occurring till the ratio has been sufficiently raised.

The shoot effect of vernalisation can proceed regardless of the time at which vernalisation is commenced (table 4.4) and consequently larger proportions of plants given vernalisation after 9 and 13 days occur in the middle region than for plants given vernalisation after 0 and 5 days (significant at the 0.001 level). It is worth noting that no quantitative effects were observed in this region indicating that the shoot effect is independent of the time at which the vernalisation is given (at least up to 13 days). These data illustrate the stability of the shoot effect since less than 10 nodes would be present at the end of vernalisation for those plants treated on day 0 and consequently 18 nodes would have to be laid down before initiation occurs. The independence of the shoot effect and time also shows that, under the growing temperatures used here, no devernalisation of this effect occurs.

The cotyledon effect of vernalisation shows up in more plants as the temperature becomes lower (significant at the 0.001 level) (table 4.5) and reaches a maximum at $3^{o}C$ where 100% of plants flowered in the low region (nodes 11-17) in the present experiment. At the completion of 38 days growth at 3,6,9,12 and $17^{o}C$ the plants had approximately 1,4,6,8 and 11

leaves expanded respectively. In the experiment where all plants were allowed to develop to the same morphological size under the different temperatures it took 38, 15, 10, 8 and 5 days to reach the stage of 2.5 to 3cm plumules (approximately 1 leaf expanded) at 3,6,9,12 and 17°C respectively. Slightly larger proportions of plants flowering in the low region were obtained under 6,9 and 12°C in the first experiment (in which all plants received the given 6,9 and 12°C in the temperature regime for 38 days) than in the second experiment (in which it was given only till 1 leaf was expanded). However a bimodal distribution of the flowering nodes still occurred further illustrating the decrease in the promotor to inhibitor ratio reaching the apex after the first foliage leaves have expanded. In both experiments the flowering node in the low region is lower in the plants exposed to 3°C than in those exposed to 6°C (significant at the 0.001 level) suggesting that a higher promotor to inhibitor ratio is produced at the lower temperature. This trend continues in the first experiment up till 9°C but not in the second experiment. Between 6 and 9° in the second experiment a significant drop in the flowering node occurs for the plants flowering in the low region. This drop probably arises since plants which have not been induced at an early age do not flower late in the low region but instead in either the middle or high regions due to the expansion of the inhibitory first foliage leaves. In the first experiment a similar drop occurs, but in this case it occurs above 9°C.

In the second experiment the only plants fitting into the middle region are two plants in the 6°C treatment (table 4.5). Two plants in each of the 12° and 17°C treatment had flowering

nodes well below those of the other plants flowering in the high region. However, these plants had slow growth rates and were not induced to flower until after they were transferred to LD conditions and should therefore be included in the high region. In the first experiment plants in the 9 and 12°C treatments flowered in the middle region (table 4.5). This is difficult to see in the 12°C treatment since the flowering nodes are quite high but, on examination of the leaves expanded data, all plants except one were shown to be induced before transfer to LD conditions indicating they should be considered as flowering in the middle region. It is possible that the plants flowering in the middle region at 9 and 12°C do so not because of a shoot effect produced by these temperatures but rather due to a higher ratio of promotor to inhibitor produced by the plant during the extended growth at low temperatures. However this seems unlikely since the plants at 12°C flowered over 15 nodes after transfer from the low temperature conditions. A quantitative effect among the plants flowering in the middle region occurs, those exposed to 9°C flowering at a lower node than those exposed to 12°C (significant at the 0.001 level). These results suggest that temperature x length of exposure is important in determining the size of a shoot effect.

The effect of the post-vernalisation growing temperature on the flowering node of L63 plants can be inferred from the variation in the response to approximately 4 weeks vernalisation in tables 4.1, 4.2, 4.4 and 4.5. Table 4.6 contains the results of an experiment designed to study this problem. The data for the unvernalised plants exposed to 10,15,20,25 and 31°C for 2 weeks are not significantly different although a few plants in the low region (nodes 11-16) occurred at 10 and 15°C and none

at 20,25 or 30°C. However, this same range of temperatures caused significant changes in the distribution of the flowering nodes of plants which had previously received vernalisation (significant at the 0.001 level using a 3 x 5 contingency X^2_8 on the data for V plants in table 4.6). The number of leaves expanded for the vernalised plants at the completion of the post-vernalisation treatment at 10,15,20,25 and 31°C were approximately 2.8, 4.3, 6.0, 6.4 and 6.2 respectively. These figures would indicate that at least in the 20,25 and 31°C treatments the post-vernalisation treatment would have extended until all the nodes in the low region had been laid down (see chapter 2). The percentage of plants in the low region drops from 90% to 4% as the post-vernalisation temperature is increased over the range 10° to 31°C (significant at the 0.001 level). Also the percentage of plants in the low region showing vegetative reversion increases from 15% to 100% (significant at the 0.001 level) and the average length of this reversion increases from 1 to 12 nodes (significant at the 0.001 level) (table 4.6). A quantitative effect also occurs within the flowering nodes of the low plants from 10,15,20 and 25°C; the higher the post-vernalisation temperature the higher the flowering node (significant at the 0.01 level). This devernalising effect by post-vernalisation temperatures appears to be a continuous effect, no critical temperature being necessary. It is suggested that this action of post-vernalisation temperature is due to a lower ratio of promotor to inhibitor being formed as the temperature increases possibly because the formative reactions of the inhibitor have a higher temperature co-efficient than those for the promotor (i.e. the same mechanism as for the cotyledon effect of vernalisation).

The proportion of plants flowering in the middle region (nodes 19-39) increases with increasing post-vernalisation temperature (table 4.6). This is primarily caused by the devernalisation of the cotyledon effect. However at 31°C 40% of plants fit into the high region and this is the clearest illustration that devernalisation of the shoot effect is also possible. Since the flowering nodes within the middle region become significantly later (at the 0.001 level) as the post-vernalisation temperature increases from 10 to 31°C it would appear that partial devernalisation of the shoot effect is possible. For this reason a critical temperature at the biochemical level is not envisaged to play a part in the devernalisation of the shoot effect, just a continuous drop in its intensity until it finally disappears in some plants (in this case at 31°C).

The devernalisation of the cotyledon effect of vernalisation does not occur if 1 weeks growth under normal short day conditions (approximately 17-25°C) is inserted between the 4 weeks vernalisation and the 2 weeks at 32°C (table 4.7, experiment 1). This result is not unexpected since after 1 weeks growth under normal conditions 4 leaves were expanded (total nodes approximately 13) and consequently the nodes in the low region had already been partially laid down. No stabilisation of the vernalisation response by the normal growing temperatures is therefore envisaged. This is supported by the fact that 86% and 100% of plants flowering in the low region in the treatments having 1 and 2 weeks at normal temperatures before being exposed to 32°C showed vegetative reversion respectively compared to 0% in the normal vernalised plant (table 4.7). All plants flowering in the low region in the treatment which received 2 weeks at 32°C immediately following vernalisation also showed

vegetative reversion, indicating that even when only 1 leaf is expanded a sufficiently promotory level of the flowering hormones can be established to ensure flowering even though the plants are receiving inhibitory growing temperatures at the time of initiation and subsequently revert to vegetative growth In the middle region significant differences (at the 0.01 level) occur between the flowering nodes of the four treatments (table 4.7, experiment 1). The latest treatment was the one receiving 2 weeks of normal temperatures prior to the high temperature treatment followed by the treatment receiving one week at normal temperature and indicates that the devernalisation of the shoot effect does not stabilise itself at intermediate temperatures. If anything, it becomes more sensitive to devernalising temperatures the later these are given (prior to the time of initiation).

The effect of light intensity on the effectiveness of devernalisation by 30°C is shown by the results of experiment 2 in table 4.7. At high intensity, devernalisation at 30°C is relatively ineffective, only reducing the proportion of plants flowering in the low region from 50% to 33% and delaying the flowering node in the middle region (nodes 18-40) by 2.7 nodes (significant at the 0.05 level). If the high temperature treatment is given to plants in complete darkness devernalisation is more effective, the proportion of plants in the low region being lowered to 10% and the flowering node in the middle region being 7 nodes later than in the plants which were not devernalised (significant at the 0.001 level). Also, 3 plants did not flower until transferred to LD (from a comparison of the flowering node and leaves expanded data). The flowering behaviour of plants receiving low intensity light during devernalisation was between those receiving complete darkness and high intensity light. These

results show a large interaction is occurring between devernalisation and light intensity but do not indicate whether this occurs because of the action of light on the gene *Sn* or through some other as yet unknown mechanism.

Vernalisation of seed during its development on the parental plant has almost exactly the same effect as 4 weeks vernalisation given immediately after planting. It resulted in a mean flowering node of $14.33 \pm .90$ and the distribution was bimodal, 9 plants flowering in the low region (nodes 11-15 in this case) and 5 plants flowering in the middle region (17-20 in this case) (table 4.8). The control plants had a mean flowering node of 42.10 ± 5.04 including 2 impenetrant plants flowering in the low region. These results indicate that vernalising temperatures given during the development of the seed on the parent plant can induce a shoot effect since only by this mechanism will L63 plants be induced to flower in the middle region. It is not clear from the present results if a response in the cotyledons is also occurring although the large proportion of plants flowering in the low region would support this view. If a response is occurring in the cotyledons it would show that hormonal balances can differ in seeds which have developed in different environments.

DISCUSSION

The results indicate that the cotyledon effect of vernalisation becomes gradually more pronounced as the temperature drops and is reversed as the post-vernalisation temperature increases. These results support the mechanism proposed on page 95 which suggested this effect acts through the different temperature coefficients for the formative reactions of the promotor and inhibitor. The stability of the shoot effect to

normal growing temperatures is also consistent with its postulated mechanism (on page 94) which suggested that the ratio of promotor to inhibitor required at the apex for flowering is permanently lowered. However, at high temperatures some devernalisation occurred, this effect increasing the closer the period of high temperature was to the time of initiation and the higher the temperature. It is suggested that this effect is, at least partly, if not entirely, caused by a general decrease in the ratio of promotor to inhibitor within the plant due to the increased temperature and the reduction of the aging response and not by reversion of the effect at the shoot apex. Determining whether this explanation is correct would require grafts to be done at various ages with devernalised plants. This would pose considerable difficulty due to the reduced vigour of devernalised plants. The difference in the effectiveness of devernalisation of the shoot effect between experiments appears to be at least partly due to the differing light intensities used in the experiments (table 4.8). Napp-Zinn (1960) and Schwabe (1955, 1957), working with *Arabidopsis* and *Chrysanthemum* respectively, have also shown that devernalisation is more effective under low light intensities. It is possible that the effect at the apex is only devernalisable at low light intensities while the devernalisation via a decreased promotor to inhibitor ratio is possible under both sets of conditions.

In neither of the experiments to determine the length of vernalisation required for a response has a plateau been reached. However, in some of the other experiments 4 weeks vernalisation has caused all plants to flower in the low region (e.g. table 4.5). This variation probably results from the slightly different post-vernalisation temperatures experienced on the trucks at different times of the year. In intact plants

Highkin (1956), Moore and Bonde (1962) and Amos and Crowden (1969) have all shown that the size of the vernalisation response in late cultivars under long photoperiods becomes larger as its duration is increased to 4 weeks. Amos (1974) showed that further vernalisation resulted in a decrease in the size of the vernalisation response. In decotyledonised plants (cotyledons removed after vernalisation) maximal promotions of the flowering nodes were observed after 2 to 3 weeks vernalisation, longer periods resulting in smaller responses (Amos and Crowden, 1969; Amos, 1974). In the present work no reduction in the size of the vernalisation response was observed in either intact or decotyledonised plants as the length of vernalisation was increased to 4 and 5 weeks respectively. The reason for this difference has not been examined although the different experimental conditions and genotypes used are possibly responsible.

Amos and Crowden (1969) using the late cv. Greenfeast showed that vernalisation was most effective when given from the start of germination, the effect gradually becoming smaller until no effect was observed after 14 days in intact plants or 10 days in plants with their cotyledons removed after the completion of vernalisation. Highkin (1956) found that in the late cv. Zelka the ability for vernalisation was lost within 5 days of the start of germination and that this was not due to the laying down of new nodes. In L63 the proportion of plants flowering in the low region drops significantly if vernalisation is left until 9 days after the start of germination, a total of approximately 12 nodes being present at this stage. It was suggested in the results that this loss of sensitivity by L63 to vernalisation arose due to the inhibitory ratio of the flowering hormones produced by the first foliage leaves, vernalisation not

being able to reduce the ratio sufficiently to cause initiation in the low region. This explanation cannot explain the results of Highkin and Amos and Crowden since a long photoperiod was used in their experiments.

The ability for a shoot effect of vernalisation to occur at the same intensity, regardless of whether the vernalisation treatment is given from the start of germination, from day 13 in intact plants or from day 21 in decotyledonised plants indicates that as long as the flowering node of a cultivar is high enough vernalisation can probably proceed at any age in peas. This statement is reinforced by the observation that plants with 20 leaves expanded can be readily induced to flower by exposure to cold temperatures, although whether this effect is due to an effect of vernalisation on the apex or through a raising of the ratio of promotor to inhibitor is not known.

Most plants with a quantitative requirement for vernalisation can be vernalised during seed germination like peas (e.g. winter cereals (Gassner, 1918); *Arabidopsis* (Napp-Zinn, 1957)). After germination many of these species show a decline in their sensitivity to cold temperatures similar to those reported in peas by Highkin (1956) and Amos and Crowden (1969). Lang (1965) suggested this may be due to a lack of storage materials. In peas this suggestion does not appear to hold, sensitivity to cold temperatures remaining throughout the life of the plant provided the flowering node of the cultivar used is sufficiently high (e.g. L63). One species with distinct similarities in its temperature responses to peas is *Vicia faba*. As in L63 it becomes slightly more sensitive to vernalising temperatures soon after the start of germination and the effect of vernalisation is accentuated by subsequent growth under warm SD conditions.

Further, warm night temperatures are inhibitory to flowering (Evans, 1959a). The ability of peas to respond to vernalisation over their entire life is distinctly different from many species with an obligate requirement for vernalisation (e.g. *Hyoscyamus niger* (Sarkar, 1958); *Lunaria biennis* (Wellensiek, 1958); *Streptocarpus wendlandii* (Oehlkers, 1956); stocks (Kohl, 1958)). The majority of this type of plant are unable to respond until a certain amount of vegetative growth has occurred causing the plants to have a biennial habit.

Previous workers (Highkin, 1956; Moore and Bonde, 1962) have only shown small devernalisation responses in peas (maximum of 3 nodes). The reason for the small size of these effects is probably the use of L type peas and long photoperiods. The temperatures capable of causing vernalisation and devernalisation in peas are similar to those found in other species (see review by Lang, 1965). Little work on the effect of varying growing temperatures has been carried out in peas. However, both Barber (1959) and Paton (1969) showed that low growing temperatures (10 to 17^{o}C) were promotory when compared to temperatures above 20^{o}C in late cultivars under 8 to 16h photoperiods. In continuous light the reverse occurs to a small degree. These results are not unexpected since in short photoperiods the ratio of promotor to inhibitor would be expected to decrease as the temperature was increased, if as suggested previously, the formative reactions of the inhibitor possess a higher temperature coefficient than those of the promotor. In continuous light, inhibitor production by the *Sn* gene is suppressed and consequently as the temperature is increased the ratio of promotor to inhibitor may rise since the formative reactions of the promotor would still be expected to have a positive

temperature coefficient.

Unlike the present results with L63, Amos (1974) could find no evidence in L24 that vernalisation could influence a seed while it was still developing in the pod. This difference has probably arisen since in Amos' experiment vernalisation was only given to the developing seeds during an 8h dark period and since L24 is not nearly as responsive to vernalisation as L63. The present result is similar to the response reported in rye by Gregory and Purvis (1938) and indicates the stability of the shoot effect of vernalisation in peas, a factor which is similar to the responses observed in other species (Wareing and Phillips, 1970).

Table 4.1 Distribution of the node of first initiated flower for L63 treated as follows: unvernalised (UV) and vernalised (V) intact plants, unvernalised and vernalised decotyledonised plants (UV- and V- respectively) and grafted in various ways (e.g. unvernalised scion and stock UV/UV). The numbers in brackets represent slow grafts. Grafts and cotyledon removal were performed when the epicotyl reached a length of 1-2 cm. The photoperiod was 8h.

Treatment	Node of first flower																					
	9 10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	49 50
UV	-	-	-	-	-	-	-	-	-	-	-	-	1	4	4	2	0	1	1	2	1
V	1	5	6	2	-	1	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
UV-	-	-	-	-	-	-	-	-	-	-	-	1	1	2	4	3	5	1	2	-	-
V-	-	-	-	-	1	18	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
UV/UV	-	-	-	-	-	-	-	-	-	-	-	2(1)	2	(2)	1(1)	0	1	3	2(1)	-	-
V/V	-	2	3	-	-	(2)	1	(1)	-	-	-	-	-	-	-	-	-	-	-	-	-
UV/V	-	2	5	1	-	-	-	-	-	-	1	0	(1)	2	0	0	0	0	0	1	-
V/UV	-	-	-	-	3(1)	1	5(3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.2 The number of L63 plants falling into the low or combined middle and high regions after being exposed to 0, 1, 2, 3 or 4 weeks vernalisation from the start of germination followed by SD conditions on the trucks.

Vernalisation (weeks)	Number of plants per class	
	Low	Middle and High
0	0	22
1	1	19
2	3	18
3	4	16
4	7	12

Table 4.3 The number of decotyledonised L63 plants falling into the middle and high regions after 0, 1, 2, 3, 4, or 5 weeks vernalisation either from the start of germination or after 24 days growth. The photoperiod was 8h. The cutoff between the classes was at node 40, the distribution of the flowering nodes being given in Appendix 1.

Vernalisation	From day 0		From day 24	
(weeks)	number of plants middle	high	number of plants middle	high
0	0	18		
1	0	18	1	14
2	0	18	8	10
3	12	8	16	2
4	15	1	14	3
5	19	0		

Table 4.4 Distribution of the node of first flower for L63 treated as follows:left unvernalised (UV) or vernalised for 4 weeks from either the start of germination (VI-4), after 4 days growth (V5-8), 8 days growth (V9-12) or 12 days growth (V13-16). The photoperiod was 8h.

Treatment	Node of first initiated flower																			
	10 11	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48 49
V1-4	1	10	3	2	-	-	2	2	2	3	5	2	2	-	-	-	-	2	-	-
V5-8	-	2	5	6	-	-	1	3	4	4	3	2	-	-	-	-	1	-	-	-
V9-12	-	-	-	-	1	-	-	2	6	8	9	2	-	1	-	-	-	-	-	-
V13-16	-	-	1	-	-	-	1	4	2	10	8	-	-	-	-	1	-	-	-	-
UV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	7	11	2

Table 4.5 Distribution of the node of first initiated flower for L63 plants given an 8h photoperiod either on the trucks at an average temperature of 17°C (17) or at a temperature of 3, 6, 9 or 12°C in growth cabinets for either the first 38 days of growth (3T, 6T, 9T and 12T respectively) or until the plumules were 2.5 to 3cm long (3D, 6D, 9D and 12D respectively). At the completion of these treatments all plants received on 8h photoperiod on the trucks.

		Node of first flower																										
Experiment	Treatment	11	12	13	14	15	16	17	18	19	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	49 50
1	17	1	1	1	2	1	-	-	-	-	-	-	-	-	1	1	-	-	1	-	3	3	2	3	5	3
1	12T	1	4	2	1	3	2	-	-	-	-	-	-	1	-	2	1	1	4	2	2	1	2	-	-	-
1	9T	-	1	4	3	7	8	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	6T	1	4	6	6	9	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	3T	5	18	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	12D	-	2	1	3	1	1	-	-	-	-	-	-	1	-	-	1	-	-	2	1	1	1	3	5	5
2	9D	-	6	2	3	1	1	-	-	-	-	-	-	-	-	-	-	-	1	1	1	2	3	2	3	3
2	6D	2	1	3	4	11	3	3	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	3D	9	18	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.6 The number of plants falling into the low, middle and high regions for L63 plants given either 4 weeks vernalisation (V) or no vernalisation (UV) followed by 2 weeks at either 10, 15, 20, 25 or 31°C before transfer to normal temperatures on the trucks. Node 17 was used as the cutoff between the low and middle regions and node 38 between the middle and high regions (for distribution see Appendix 1). The photoperiod was 8h. The percentage of plants flowering in the low region which showed vegetative reversion and the average number of nodes of this reversion are indicated.

	Temperature	Number of Plants			% Reversion	Av. length of Reversion
	°C	Low	Middle	High		
UV	10	2	1	15	0	-
	15	1	0	13	0	-
	20	0	0	15	0	-
	25	0	0	13	0	-
	31	0	0	11	0	-
V	10	26	3	0	15	1.0
	15	26	3	0	46	2.2
	20	25	4	0	88	9.1
	25	10	12	0	100	9.8
	31	1	13	9	100	12.0

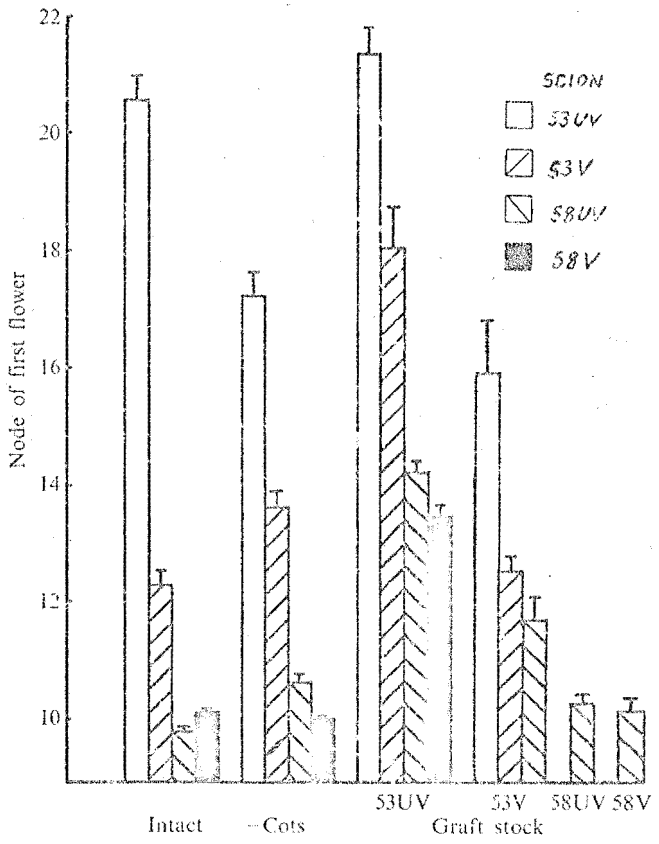
Table 4.7 Distribution of the node of first initiated flower in 2 experiments using L63 and an 8h photoperiod. In the first all plants received 33 days of vernalisation followed by SD conditions on the trucks which were either not interrupted (treatment C) or interrupted after 0, 1 or 2 weeks by 2 weeks at 32°C (designated treatments 0,1 and 2 respectively). The percentage of vegetative reversion amongst plants flowering in the low region (11-16) is indicated. In the second experiment all plants received 32 days of vernalisation followed in most by 9 days at 30°C in either complete dark (D), 8h of weak fluorescent light (L) or 8h of high intensity light from a mixed incandescent-fluorescent source (H) before transfer to an 8h photoperiod on the trucks. One treatment (V) was not exposed to the high temperature, being transferred to the trucks immediately after vernalisation.

Experiment	Treatment	Node of first flower 11	12	13	14	15	16	17	18	19	20	22	24	26	28	30	32	34	36	38	40	42	44	45 46	Percent Reversion
1	C	2	9	6	4	1	2	-	-	2	-	1	-	-	1	-	-	-	-	-	-	-	-	-	0
1	O	1	2	3	-	3	1	-	1	-	2	8	5	2	-	-	-	-	1	-	-	-	-	-	100
1	1	2	2	11	7	1	-	-	-	-	1	1	1	-	-	-	1	-	-	-	-	-	-	-	86
1	2	3	5	5	9	2	1	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	100
2	V	1	2	3	4	1	1	1	1	1	1	5	2	2	1	-	-	-	-	-	-	-	-	-	-
2	H	3	3	2	-	-	-	-	-	1	2	2	3	1	3	4	-	-	-	-	-	-	-	-	-
2	L	2	1	1	-	-	-	-	-	-	1	1	8	1	1	3	2	-	-	-	-	1	1	-	-
2	D	-	-	1	1	-	-	-	-	-	-	-	-	5	4	1	3	2	2	1	-	-	-	1	-

Table 4.8 Distribution of the node of first initiated flower for L63 plants grown on the trucks from seeds that were exposed to 7.5°C or to normal growing temperatures (approx. 20°C) from the time of fertilisation to maturity. The photoperiod was 8h.

	Node of first flower																				
TREATMENT	11	12	13	14	15	16	17	18	19	20	22	24	26	28	30	34	38	42	46	50	54
7.5°	4	3	2	0	1	-	1	1	1	2	-	-	-	-	-	-	-	-	-	-	-
20°	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4	3

Fig. 4.1. Mean node of first initiated flower (+S.E.) for intact, decotyledonised (-cots) and grafted plants of lines 58 *(lf e sn hr)* and 53 *(lf e Sn hr)* either vernalised (V) or unvernalised (UV). Grafting and cotyledon removal were performed when the epicotyl reached a length of 1-2 cm. The photoperiod was 8h.


Node of first flower
22
20
18
16
14
12
10
SCION
53UV
53V
58UV
58V
53UV
53V
58UV
58V
Intact
Cots
Graft stock

CHAPTER 5

The Effect of Age on the Gene *Sn* and the Site of Action of the Gene *Hr*.

INTRODUCTION

Since late cultivars of peas flower between nodes 20 and 35 under SD conditions it would appear that the effect of the *Sn* gene is reduced as the plant ages. Haupt (1969) and Köhler (1965) have referred to this response as autonomous determination. Murfet (1971b) suggests this response occurs due to either the switching off of the gene *Sn* or the destruction of its product. The response appears to occur in the expanded leaves or mature stem since Haupt (1954) has shown that when grafts were performed between young scions and stocks of varying ages of the L cultivar Alderman (possible flowering genotype of *Lf Sn hr* (Murfet, 1976)) the flowering node decreased as the age of the stock increased. Although this evidence is not conclusive since some of the stocks may have flowered prior to grafting due to the use of a fairly long photoperiod (14-16h), it does suggest that the site of the aging response is the same as the site of action of the *Sn* gene (chapter 3). The gene *Hr* is a modifier of *Sn* which, Murfet (1973a) suggests, acts by reducing the effect of age on the *Sn* gene rather than by increasing its output of inhibitor. This hypothesis is supported by the evidence presented in chapter 3 which shows that the genotypes *lf e Sn hr* and *lf e Sn Hr* do not show different sensitivities to LD cycles until after three weeks growth. The site of action of the gene *Hr* has not been examined but if *Hr* directly affects the aging response which in turn acts by reducing the activity of the *Sn* gene it might be expected to be in the expanded leaves and shoot. To examine this question grafts between the genotypes *lf e Sn hr* and *lf e Sn Hr* were performed when

the plants had approximately 8.5 leaves expanded. To clarify the site of the aging response under SD conditions grafts were also performed between old and young plants of genotype *lf e Sn hr*.

The genotype *lf e sn Hr* has been examined by Murfet (1973a) and is reported to possess an ED phenotype, although under poor growing conditions it has distinct EI tendencies. The three genotypes *lf e sn hr* (ED), *lf e sn Hr* and *lf E Sn hr* (EI) were examined in order to further clarify the phenotype of the genotype *lf e sn Hr* and to allow the site of action and effect of the gene *Hr* on a *sn* background to be examined.

The role of the foliage leaves in the control of flowering in peas has not been clearly determined in the literature. Paton (1967, 1968) has shown that a definite leaf requirement exists for flowering in the late cultivar Greenfeast and that this requirement is raised by shortening the photoperiod or increasing the temperature. This would be anticipated if inhibitor production can occur in the foliage leaves as suggested by Murfet (1971a, 1973b) since the results in chapters 3 and 4 indicate that shorter photoperiods and higher temperatures result in decreased promotor to inhibitor ratios. However, Sprent (1966) has suggested that the leaf area of a plant cannot affect flowering in a quantitative manner and has presented data from defoliation experiments supporting her claim. During the present study experiments using defoliation and grafting techniques were designed to clarify the previously contradictory results of Paton (1967) and Sprent (1966) and to show whether the aging response which is shown to occur in the expanded leaves (and possibly mature

stem) occurs due to each leaf becoming more promotory with age or to the later formed leaves being more promotory than the first formed leaves.

Controversy has existed for many years over the reason for the promotion of the flowering node in decotyledonised late varieties. Barber and Paton (1952), Paton and Barber (1955), Johnston and Crowden (1967) and Amos and Crowden (1969) have suggested it was caused by the removal of a source of inhibitor while several authors (e.g. Haupt, 1954, 1969 ; Köhler, 1965; Murfet, 1973b; Amos, 1974; Collins and Wilson, 1974b) have suggested it is caused as a result of the reduced rate of growth of the decotyledonised plants. Murfet (1973b) presents evidence which would suggest that, at least in some late flowering genotypes under SD conditions, cotyledon removal would result in a lower ratio of promotor to inhibitor being present in the plant soon after cotyledon removal. Amos (1974) suggests the promotion is due to a reduced number of nodes in apical bud and as a consequence of this hypothesis one must assume a distinct number of leaves need to be expanded before initiation can occur. Collins and Wilson (1974b) suggest that since the time of initiation is delayed by cotyledon removal in the late cv. Greenfeast, the promotion of the flowering node is unimportant as it does not represent a valid measure of the change to the reproductive state. This statement assumes that chronological age is of utmost importance to the plant and that the physiological age (as represented by the number of leaves expanded and the total number of nodes) is relatively unimportant in determining the onset of flowering. Murfet (1973b) suggests that the chronological and physiological ages of the plant get out of step and this leads to the phasing out of *Sn* activity at a

lower node. The present work set out to determine whether the chronological or physiological age was most important in determining the sensitivity of the genotype *lf e Sn Hr* to LD cycles and whether the explanation of Amos (1974) could account for the entire decrease in the flowering node of a decotyledonised L type line under SD.

MATERIALS AND METHODS

Determination of the Effect of the Gene *Hr* on an *sn* Background.

A factorial design was used. It consisted of the 3 lines, L68 *(lf e sn hr)*, L64 *(lf e sn Hr)*, and L60 *(lf E Sn hr)* and the three treatments, intact plants, cotyledons removed on day 5 (decot) and the cotyledons removed after 24 hours imbibition (embryos). The embryos were grown in test tubes on White's nutrient agar until 5 leaves were expanded and then transferred to the normal growing medium. The characters FI, FT, FP and TNE were scored. Fifteen plants were grown per treatment with the exception of embryos where about 20 plants were used to compensate for the losses caused by fungal attack on the young plants. The photoperiod was 8h. The results are contained in table 5.1.

Site of Action of the Gene *Hr*.

The two lines, L53 *(lf e Sn hr)* and L63 *(lf e Sn Hr)*, were grown under an 8h photoperiod on the trucks and the grafts 53/53, 53/63, 63/53 and 63/63 performed as indicated in chapter 2. At the time of grafting (day 28) there were between 7 and 10 leaves expanded. Twenty-four grafts were attempted per treatment, the number surviving ranging from 4 to 6. The results are contained in table 5.2. The flowering node of grafts refers to that of the scion counting from its own cotyledons as zero. Impenetrant plants were excluded from the analysis.

Site of Action of the Aging Response in Genotype *1f e Sn hr*.

Grafts were carried out between L53 plants of three different ages. Old plants (O) had 24 days growth before grafting and at this stage had an average of 18.33±0.26 nodes laid down and 8.45±0.22 leaves expanded (from a sample of 12 plants). No flower buds were observed in any of the apices. Young plants (Y) had only 5 days growth before grafting, their plumules being 1 - 2cm long. The oldest plants (F) were 47 days old at the time of grafting and from a sample of 12 plants had an average of 23.33±.31 nodes laid down and 12.89±.15 leaves expanded. Eight of these twelve plants had flower buds visible in their apices at the time of dissection. As well as intact plants of each age, 28 grafts of the following five types were performed:- Y/Y, Y/O, O/Y, Y/F and O/O. The photoperiod was 8h. The results are contained in table 5.3.

Mechanism of the Aging Response in Genotypes *1f e Sn hr* and *1f e Sn Hr*.

L53 plants *(1f e Sn hr)* were grown under an 8h photoperiod on the trucks until day 26 (8.81±.13 leaves expanded and 18.12±.23 nodes laid down) at which time they were either left intact or had the leaves (leaflets, stipules and petioles)removed at nodes 6 to 9, 3 to 7 or 3 to 9 (all expanded leaves). One group of plants was defoliated at nodes 6 to 9 and at subsequent nodes as they expanded until flower buds were visible. It should be noted that nodes 1 and 2 possess only small scale leaves. Twenty four plants were grown per treatment, the results being tabulated in table 5.4. A one-way analysis of covariance was performed between the flowering nodes and the number of leaves expanded after 45 days enabling adjusted flowering nodes to be obtained. This technique allows a primary adjustment to be made for different growth rates.

In a further experiment, 4 day old scions of L53 were grafted onto either 4, 20 or 37 day old stocks using the grafting technique described in chapter 2. The stocks were cut between nodes 0 and 1 for 4 day old plants, 5 and 7 for 20 day old plants and one group of 37 day old plants and nodes 10 and 13 for a second group of 37 day old plants. Only twelve intact plants and grafts with 4 day old stocks were grown. Twenty four grafts of each of the other 3 types were performed, a low success rate being obtained with 37 day old stocks cut between nodes 5 and 7 due to the "woody" nature of the stem at this time. It should be noted that the cotyledons were dead before grafting in the 20 and 37 day old stocks, but that all foliage leaves were alive. The results are contained in table 5.5.

In an experiment with genotype *lf e Sn Hr*, L63 plants were grown under an 8h photoperiod on the trucks until 29 days old (9.43±.09 leaves expanded). Groups of 72 plants were then either left intact or completely defoliated at nodes 3 to 10 inclusive (all expanded leaves), nodes 3 to 8 or nodes 5 to 10. 18 plants of each treatment were then exposed to either 1, 2 or 3 LD cycles or left continuously under SD conditions. When about 45 leaves were expanded the plants were transferred to the apron to mature. The results are recorded in table 5.6.

Effect of Chronological Age on the Sensitivity of Plants of Genotype *lf e Sn Hr* to LD Cycles.

Two groups of L63 plants were planted 21 days apart under an 8h photoperiod on the trucks. In order to alter the relationship between chronological and physiological age half the younger plants (young) and all the older plants (old) had their cotyledons removed 5 days after the start of germination (young 5

and old 5 respectively). The remainder of the young plants had their cotyledons removed on day 27. On day 27 for the young plants and day 48 for the old plants groups of 12 plants of each treatment were exposed to 1, 2, 3 or 4 LD cycles or left under continuous SD conditions. The young 5 and old 5 treatments also received 5LD cycles. The results are contained in table 5.7.

The Relationship Between the Number of Nodes in the Apex and the Flowering Node in Genotype *lf e Sn hr*.

Plants of L53 were grown under an 8h photoperiod on the trucks and were either left intact or had their cotyledons removed on day 5. Twenty one plants of each treatment were grown, 6 plants of each type being dissected when leaf 10 was just coming free of the stipules and the total number of nodes recorded This developmental stage coincided with the time at which initiation was occurring in the decotyledonised plants. The results are contained in table 5.8.

RESULTS

As previously shown by Murfet (1971a, 1973b) intact plants of genotype *lf E Sn hr* (L60) show considerably increased flowering time, node of first pod and total number of leaves expanded values when compared to genotype *lf e sn hr* (e.g. L68) under an 8h photoperiod (table 5.1). Although both possess flowering node values within the early region (L60 is, however, significantly later than L68 at the 0.001 level) these characteristics allow distinct classification of these genotypes into the ED and EI classes respectively (Murfet, 1971a). Intact plants of the genotype *lf e sn Hr* (e.g. L64), although flowering at the same node as L68, show flowering time, node of first pod and total number of leaves expanded values intermediate between those of L68 and L60 confirming the previous results of

Murfet (1973a). Cotyledon removal in the lines 68, 64 and 60 under SD conditions leads to delays in the flowering node of 0.56 (not significant), 4.20 and 7.56 nodes (both significant at the 0.001 level) respectively. The results for L64 are most easily interpreted as suggesting *Hr* is active in the shoot but not in the cotyledons since with the cotyledons intact and therefore forming the bulk of the plant tissue over the first 2 weeks of growth the promotory ratio of the flowering hormones coming from them would swamp any inhibitory effect coming from the developing shoot. However, in decotyledonised plants the ratio of promotor to inhibitor reaching the apex is determined solely by the developing shoot (except for any contribution from the cotyledons prior to their removal on day 5) and consequently the flowering node is delayed. The effect of *Hr* in the shoot is still, however, observed in intact plants since the rate of floral development and senescence are delayed in genotype *lf e sn Hr* when compared to genotype *lf e sn hr*. This interpretation is consistent with the results in chapter 3 which show that *Hr* does not affect the number of LD cycles required for induction until after 3 weeks growth on a *lf e Sn* background. The results for L60 support the hypothesis that the gene *E* causes a more promotory promotor to inhibitor ratio to be produced by cotyledons carrying *Sn* and that the gene *E* does not appear to be active in the shoot (Murfet, 1971c, 1973b). In continuous light, intact L64 plants flower 0.68 nodes later than L68 plants (significant at the 0.001 level) while cotyledon removal leads to a small but insignificant promotion of the flowering node in both lines. This last result indicates that in L64 the effect of decotyledonisation is photoperiodic since a substantial delay was observed under SD conditions. It is not clear whether the *Hr* gene in L64 is acting by delaying the effect of age on the *Sn* locus as

postulated by Murfet (1973a) or by another mechanism. If it is acting by the first mechanism the gene *sn* must be a leaky mutant as suggested by Murfet (1971c), Amos (1974) and Reid and Murfet (1975a). The first alternative would seem the more likely since the *Sn* locus has been shown to exhibit a photoperiod effect (Barber, 1959) and this effect is even apparent to a small extent in decotyledonised plants of genotype *lf e sn hr* (significant at the 0.001 level, see L68, table 5.1). Consequently it does not seem necessary to postulate a second gene *(Hr)* capable of controlling a photoperiod effect.

Embryo culture of L68 and L64 plants under both photoperiods led to a delay of 2.0 to 2.5 nodes in the flowering node when compared to the decot treatment. As this extra delay exhibits no photoperiod response it seems unlikely to involve the *Sn* locus. This delay could be an indirect effect due to the build up of ethylene in the test tubes during embryo culture as ethylene is shown to delay the flowering node of the ED variety L58 by up to 4.7 nodes in chapter 7. L60 plants raised from embryos flower 2.1 nodes earlier than the L60,decot treatment. This anomaly could be explained by the very poor growth rate of this treatment (even compared to the other embryo treatments) since poor growth has previously been shown to reduce the flowering node of plants flowering in the late region (Reid and Murfet, 1974a).

When grafts were performed between L63 *(lf e Sn Hr)* and L53 *(lf e Sn hr)* plants with approximately 9 leaves expanded one result stood out. The 63/53 grafts flowered 30 nodes earlier than the 63/63 grafts (significant at the 0.001 level) indicating that the principal site of action of *Hr* is in the

mature stem or expanded leaves (table 5.2). However the 63/53 grafts were still significantly later (at the 0.001 level) than the 53/53 grafts. The 53/63 grafts flowered at a similar node to 63/53 grafts but were not significantly later than 53/53 grafts due to the large variance of the 53/63 treatment which may be attributed to the flowering of two of the L53 scions prior to the graft union becoming fully operative. The site of action of *Hr* (from the data in tables 5.1 and 5.2) therefore appears to be in the developed shoot and leaves and not in the cotyledons or at the apex. L53 intact plants flowered significantly later (at the 0.001 level) than the 53/53 grafts, possibly due to the incomplete operation of the graft union or the reduced growth rate caused by grafting which allowed aging processes to continue whilst growth was retarded.

The site of the aging response would appear to be in the mature stem or leaves since young scions of L53 (plumules 1-2 cm long) grafted onto old stocks (O) of L53(possessing between 8 and 10 expanded leaves) flowered 7.86 nodes earlier than self-grafted old plants (significant at the 0.001 level) (table 5.3). This change could not be due to some form of secondary induction brought about by previous flowering of the stocks as the apices of 12 stock plants were dissected and no flower buds were observed. When young scions were grafted onto stocks that had mostly initiated (had 12-14 leaves expanded) they flowered 1.27 nodes earlier than when grafted onto O stocks (significant at the 0.001 level). This promotion could be explained by the continued aging of the stocks, no rapid change in the level of the flowering hormones at the time of initiation being indicated. No significant difference in the flowering node was observed between self grafts made with young plants

or old plants. Old scions grafted onto young stocks were significantly delayed compared to old/old grafts (significant at the 0.001 level) indicating that 5 day old cotyledons produce a more inhibitory level of the flowering hormones than shoots with between 8 and 10 leaves expanded. These results support those of Haupt (1954) and show that the site of the aging response and the site of action of *Hr* are similar to that of the site of activity of the *Sn* gene. This is consistent with the hypothesis that *Hr* blocks the aging response, and that this response is due to a drop off in the activity of the *Sn* gene in the leaves (Murfet, 1971b, 1973a). *Hr* could be operating at the gene level by producing a substance which can combine with a repressor of the *Sn* gene, the amount of repressor normally increasing with age. Other possible mechanisms could occur and without a knowledge of the chemical nature of the products of the two genes no definite answer regarding the nature of the mechanism can be given.

When the lower leaves (leaves 3,4 and 5) are removed from L53 under SD conditions the flowering node is increased relative to the intact plants while if the higher expanded leaves (leaves 6 to 9) are removed it is decreased (table 5.4). Continued removal of newly expanded leaves on plants defoliated initially from nodes 6 to 9 or removal of all expanded leaves at one time also resulted in a lowering of the flowering node (table 5.4). From a one-way analysis of variance these results were shown to be significantly different at the 0.001 level. However, the defoliation treatments also alter the rate of vegetative growth and vigour of the plants and this has been reported to affect the flowering node of late cultivars (Haupt, 1969; Reid and Murfet, 1974a). In order to make

a primary adjustment for this altered rate of growth a one-way analysis of covariance was performed between the flowering node and the number of leaves expanded after 45 days' growth. The adjusted mean flowering nodes for the five treatments were still significantly different (at the 0.001 level) suggesting that the altered flowering nodes caused by the defoliation treatments were not entirely due to altered rates of vegetative growth. It would seem that the lower leaves are slightly more promotory than the later formed leaves at the time of defoliation. This would suggest that each leaf goes through a cycle from inhibitory to promotory as it ages. This view is reinforced by the fact that 4 day old L53 scions grafted onto 20 day old L53 stocks, cut between nodes 5 and 7, flower 5.6 nodes later than similar grafts where the stock is 37 days old. (table 5.5). With 37 day old stocks, grafts performed just below the apical bud (between nodes 10 and 13) only flowered slightly later than those performed between nodes 5 and 7 (not significant) indicating that the later formed leaves are not particularly inhibitory. This may suggest that as well as each leaf producing a higher ratio of promotor to inhibitor as it ages the later formed leaves may start off with a slightly higher ratio than the earlier formed leaves. This may also explain the rather small responses to defoliation observed in the present experiments and previously reported by Sprent (1966a) since the two responses would oppose each other.

Two groups showed up when intact L63 plants, or L63 plants with leaves 3 to 8, 5 to 10 or 3 to 10 (all expanded leaves) removed, were exposed to continuous short days or 1,2 or 3 LD cycles, (table 5.6). Although the intact plants did show slightly higher percentages of flowering plants after

1 and 2 LD cycles than did the plants with leaves 3 to 8 removed, the difference was not significant. Similarly plants with leaves 5-10 removed showed slightly higher percentages (not significant) of flowering plants after 2 and 3 LD cycles than did those with leaves 3 - 10 removed. This latter treatment had very small numbers of surviving plants due to the severe nature of the defoliation. The first two treatments, however, showed much higher percentages of flowering plants after 2 LD cycles than did the latter 2 treatments. This difference between intact plants and plants with all their expanded leaves removed (leaves 3-10 removed) could simply reflect a reduced ability by the latter type to perceive the photoperiod or that the plant could not produce sufficient promotor during the continuous light treatment to alter the original inhibitory ratio of promotor to inhibitor to the same extent as in intact plants. Although the plants with leaves 5-10 removed did have a smaller residual leaf area than did plants with leaves 3-8 removed, it seems unlikely that the difference (significant at the 0.001 level) in the percentage of flowering plants would remain almost as large as between the intact and completely defoliated treatments due to this difference alone. I suggest that the difference is also contributed to by the fact that the lower leaves in this genotype *(lf e Sn Hr)* produce a lower ratio of promotor to inhibitor under short day conditions than do leaves formed at a later stage. This result in L63 is different to that observed in L53 since no aging of an individual leaf is indicated. It appears the gene *Hr* may be responsible for this difference between L53 and L63. However, the genotype *lf e Sn Hr* could still become more sensitive as it ages due to the later formed leaves being slightly more promotory than the earlier formed leaves as appears to occur

in both genotype *lf e Sn hr* and *lf e Sn Hr*. It seems likely that at least the aging response being affected by *Hr* is due to a drop in inhibitor levels and not an increase in promotor levels since *Hr* is a specific modifier of *Sn* (Murfet, 1973a).

The results in table 5.7 come from an experiment designed to examine whether chronological age or the stage of physiological development is more important in determining the sensitivity of L63 plants to various numbers of LD cycles. Plants of the same chronological age as the control plants (treatment young, 27) but possessing a reduced amount of physiological development were produced by decotyledonising a group of plants on day 5 (young 5). The young 5 treatment was less sensitive to LD cycles than the young 27 treatment indicating that chronological age cannot be the only factor affecting the sensitivity of the genotype *lf e Sn Hr* to LD cycles. However, this result does not mean that chronological age does not have some effect on the sensitivity of the plant. In order to try and obtain plants at the same stage of physiological development but possessing a different chronological age from the young 27 treatment a group of plants were planted 21 days before these plants and decotyledonised on day 5 (treatment old 5). Unfortunately at the time of treatment the old 5 treatment possessed significantly (at the 0.001 level) more expanded leaves than the young 27 treatment. The old 5 treatment was slightly more sensitive to 1 LD cycle than the young 27 treatment. This difference could be attributed to the different stages of physiological development in the 2 treatments and need not reflect the effect of chronological age. The results therefore suggest that chronological age is

not of prime importance in determining the sensitivity of L63 to LD cycles and would support the idea that a leaf requirement exists for flowering in peas in a particular set of environmental conditions as suggested by Paton (1967).

The suggestion by Amos (1974) that the difference in the number of nodes in the apex between intact and de-cotyledonised plants can explain the entire promotion of the flowering node caused by cotyledon removal is shown not to be true in L53 under SD (table 5.8). Although the two treatments do possess different numbers of nodes in the apex (significant at the 0.001 level) this difference could only account for 1.5 nodes of the total promotion of 5.5 nodes in the flowering node.

DISCUSSION

The classification of the genotype *lf e sn Hr* as ED would seem inappropriate since under good summer growing conditions this genotype is closer to the genotype *lf E Sn hr* than to the genotype *lf e sn hr* in terms of the total number of leaves expanded and the flowering node shows a large delay after cotyledon removal (4.2 nodes). Murfet (1973a) has shown similar tendencies of this genotype towards the EI phenotype, particularly under poor growing conditions, and consequently I suggest that at least where physiological experiments are concerned it is not included in any of the present phenotypic classes. This raises the question of the usefulness of the phenotypic classes as a system of classifying the variation in the flowering behaviour observed in peas. It was undoubtedly very useful at the start of the major crossing programme performed by Marx (1969) and Murfet (1971a) and allows other workers to relate their own results to those of the above

authors. However, sufficient genetic variation appears to exist in peas to allow the selection of intermediates between all the classes (e.g. I64 between ED and EI; L65 between ED and L (Murfet and Reid, 1974); L61a gives plants fitting into both the EI and L classes (chapter 7) etc.). It may now be more appropriate to describe the responses of new genotypes of peas by comparing their behaviour to that of the lines already reported in the literature. This approach has been used with great success by Murfet (1975) and, provided the reported genotypes are readily available, will result in a much clearer classification of the flowering behaviour of new genotypes.

The suggestion that the gene *sn* is a leaky mutant, as well as explaining the photoperiod effect observed in decotyledonised plants of the genotypes *lf e sn hr* and *lf e sn Hr* and the action of the gene *Hr* on a *sn* background would also explain, at least partly (Rowberry, unpub.), the small photoperiod effect observed by Murfet and Reid (1974) in intact L65 plants *(Lf e sn hr)*. This effect is possibly observed in L65 plants since this genotype has a very late group of polygenic modifiers and does not initiate until well after it has come through the ground. Most lines carrying *sn* initiate before or very soon after emergence and would therefore not have time to respond to the photoperiod.

Since it appears that each leaf in genotype *lf e Sn hr* becomes more promotory as it ages it would be anticipated that for a particular treatment a definite leaf requirement would exist in late cultivars as suggested by Paton (1967). However, the suggestion by Sprent (1966) that foliage leaves are not

involved in the photoperiod response in a quantitative fashion is easily reconciled with Paton's view when it is realised that as well as each leaf becoming more promotory with age, later formed leaves may start with a more promotory level of the flowering hormones than the earlier leaves. These two responses oppose each other, resulting in the removal of any leaf or group of leaves tending to have only a small effect on the flowering node. The differences between the results of Paton and Sprent probably arise due to the different severities of the defoliation treatments used by these two workers. The reason for the delays observed by Paton after defoliation probably result from the increased effect of the inhibitor produced in the shaded cotyledons when leaves exposed to a 19h photoperiod are removed. In other species it has been shown that the later formed leaves are often more promotory than the earlier leaves (e.g. *Lolium* (Evans, 1960b); *Sinapis* (Lang, 1965); *Bryophyllum* (Zeevaart, 1962) and *Perilla* (Zeevaart, 1958)). The observation that each leaf becomes more promotory as it ages does not appear to have been reported although a peak has been observed in *Xanthium* (Lang, 1965). This difference in the mechanism of the increase in sensitivity with age between peas and other species again illustrates how evolution may solve the one problem in different ways in separate groups.

The reason for a promotion of the flowering node after cotyledon removal in late varieties in SD would not appear to be due to the removal of a source of inhibitor (Murfet, 1973b) although this may well account for part of the promotion in long photoperiods since the buried cotyledons would then be the major source of inhibitor. Although a portion of the effect could be attributable to a

reduced number of nodes in the apex when the leaf requirement is met, the majority of the promotion, at least in L53 under SD conditions, would appear to be caused by some other mechanism. Since chronological age does not appear to be of major importance in determining the sensitivity of the genotype *lf e Sn Hr* to LD cycles and the effect of self grafting on the flowering node is usually small in late cultivars even though the growth rates are markedly changed (Murfet, 1971a, chapter 4), the arguments of Collins and Wilson (1974b) explaining the promotion purely in terms of chronological age would seem invalid. The use of the time to initiation as a measure of the change from vegetative to floral development as suggested by Collins and Wilson (1974b) would not appear an improvement over the flowering node due to this lack of importance of the chronological age, although both characters may need to be considered in some circumstances. I suggest the promotion caused by cotyledon removal in late cultivars comes about due to several reasons. Firstly, the chronological age and physiological development of the leaves may get out of step with each other resulting in a change to the production of a promotory level of hormones at an earlier stage of physiological development (analogous to the explanation of Murfet 1973b), secondly a reduced number of nodes in the apex results in earlier flowering once the leaf requirement is met and thirdly the removal of a source of inhibitor provided the shoot is exposed to a long photoperiod.

Table 5.1 Mean node of first initiated flower (FI) ± S.E., flowering time (FT), node with first pod (FP) and total number of leaves expanded (TNE) for lines 68 *(lf e sn hr)*, 64 *(lf e sn Hr)* and 60 *(lf E Sn hr)* exposed to either an 8h photoperiod or continuous light. The plants were either left intact or decotyledonised on day 0 (Embryo) or day 5 (Decot).

		8 Hour Photoperiod						Continuous Light			
		L68		L64		L60		L68		L64	
CHARACTER	TREATMENT	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n
FI	Intact	9.80 ± .11	15	9.87±.13	15	11.13 ±.16	15	9.47±.13	15	10.15±.10	13
FI	Decot	10.36 ± .27	14	14.07±.28	15	18.69 ±.43	13	9.15±.10	13	10.07±.12	15
FI	Embryo	12.87 ± .17	23	16.45±.31	11	16.59 ±.44	17	11.77±.20	13	12.11±.18	18
FT	Intact	34.40 ± .19	15	41.33±.84	15	59.67±2.14	15	29.13±.36	15	30.46±.54	13
FP	Intact	9.80 ± .11	15	12.73±.32	15	18.47 ±.52	15	9.47±.13	15	10.15±.10	13
TNE	Intact	14.40 ± .21	15	20.13±.22	15	23.46 ±.51	13	12.86±.18	14	13.83±.24	12

Table 5.2 The mean node of first initiated flower ± S.E. for lines 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)* and the grafts 53/53, 53/63, 63/53 and 63/63. The photoperiod was 8h and the grafts were performed after 28 days growth (about 9 leaves expanded).

STOCK	SCION L53 $\bar{x}$ ± S.E.	n	SCION L63 $\bar{x}$ ± S.E.	n	INTACT $\bar{x}$ ± S.E.	n
L53	18.75± .25	4	20.50± .43	6	20.94± .40	16
L63	20.33± 1.26	6	50.33± 2.11	6	49.88± 1.03	17

Table 5.3 The mean node of first initiated flower ±S.E. for grafts between L53 plants *(lf e Sn hr)* of three ages; young plants (Y) being 5 days old, old plants (O) being 24 days old and flowering plants (F) being 47 days old at the time of grafting. The photoperiod was 8h.

	SCION				INTACT	
	Y		O			
STOCK	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
Y	20.72 ± .29	25	22.86 ± .53	22	22.60 ± .37	10
O	12.86 ± .43	21	20.33 ± .11	18	22.86 ± .59	7
F	11.59 ± .18	23	———		20.60 ± .68	5

Table 5.4 The mean node of first initiated flower (FI)±S.E., number of leaves expanded after 45 days growth (LE)±S.E. and mean adjusted flowering node (Adj. FI) ± S.E. for L53 *(lf e Sn hr)* plants either left intact or defoliated at nodes 6 to 9, 3 to 7 or 3 to 9 on day 26 or defoliated at nodes 6 to 9 on day 26 and at subsequent nodes as they expanded (cont.). The photoperiod was 8h and $n \geqslant 19$.

CHARACTER	INTACT $\bar{x}$±S.E.	6 to 9 $\bar{x}$±S.E.	3 to 7 $\bar{x}$±S.E.	3 to 9 $\bar{x}$±S.E.	CONT. $\bar{x}$±S.E.
FI	22.83±.35	21.61±.22	23.60±.57	21.42±.32	21.32±.20
LE	17.35±.23	16.43±.22	16.65±.31	14.53±.26	15.56±.15
ADJ. FI	21.93±.43	21.39±.30	23.22±.33	22.61±.57	21.75±.35

Table 5.5 Mean node of first initiated flower ± S.E. for L53 plants exposed to an 8h photoperiod and either left intact or grafted in various ways. The scions were all 4 days old at the time of grafting while the stocks were either 4, 20 or 37 days old (treatments Y, M and O respectively). The grafts were performed either between nodes O and 1 (Y), nodes 5 and 7 (M5 and 05) or nodes 10 and 13 (010) on the stock plants.

05		010		M5		Y		INTACT	
$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
11.71±.36	7	12.00±.26	10	17.27±.53	15	21.43±.37	7	24.00±1.38	11

Table 5.6 L63 plants *(lf e Sn Hr)* were either left intact or defoliated at either nodes 3 to 8 (-3 to 8), 5 to 10 or 3 to 10 after 29 days growth. The percentage of plants induced to flower (%) under continuous SD conditions (SD) or after 1,2 or 3 LD cycles (first cycle 32h, then multiples of 24h of light) is indicated along with the number of plants used (n).

	SD		1LD		2LD		3LD	
TREATMENT	%	n	%	n	%	n	%	n
INTACT	0	12	15	13	100	15	100	11
-3 to 8	0	11	0	14	92	12	100	11
-5 to 10	0	11	0	12	27	15	100	14
-3 to 10	0	3	0	7	20	5	40	5

Table 5.7 L63 plants were decotyledonised on day 5(5) or day 27(27). Either 27 day old (young) or 48 day old (old) plants were then exposed to either continuous SD conditions (SD) or 1,2,3,4 or 5 LD cycles (first cycle 32h, then multiples of 24h light). The percentage of plants induced to flower is shown for each treatment (%) as well as the mean number of leaves expanded (LE) at the commencement of the treatment and the number of plants tested (n).

TREATMENT	SD		1LD		2LD		3LD		4LD		5LD		LE
	%	n	%	n	%	n	%	n	%	n	%	n	
YOUNG 27	0	6	25	8	100	11	100	8	100	10	-	-	9.11±.13
OLD 5	0	6	64	11	100	12	100	13	100	12	100	8	12.29±.17
YOUNG 5	0	6	0	10	0	11	25	8	70	10	70	10	6.59±.10

Table 5.8 The mean node of first initiated flower (FI) ± S.E. and number of nodes present when leaf 10 came free of the stipules (TN) ±S.E. for L53 plants *(1f e Sn hr)* exposed to an 8h photoperiod and either left intact or decotyledonised on day 5 (Decot 5).

CHARACTER	INTACT		DECOT 5	
	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
FI	23.00 ± .83	12	17.50 ± .49	14
TN	19.17 ± .17	6	17.67 ± .33	6

CHAPTER 6

The development of a bioassay for compounds affecting flowering.

INTRODUCTION

Before a search can be commenced for a compound or an extract which could mimic the action of a specific gene a test system needs to be devised which can respond readily to a single dose of the required substance. Murfet (1971b, 1973b) has reported that the penetrance of the gene *Sn* is not complete in the genotype *lf e Sn hr*, the penetrance varying from 0.5 to 0.98 The impenetrant plants are phenotypically similar to EI plants while the penetrant plants are L types. He has shown that a set of penetrance modifiers exist which act in the cotyledons to alter the balance of flower promotor to inhibitor reaching the apex. The penetrance can be significantly varied by small environmental variations and by the removal of 1 or both cotyledons suggesting that at the time nodes 10-16 are laid down (the nodes at which impenetrant plants flower) the ratio of promotor to inhibitor is close to the threshold for flowering. A line of plants having a penetrance between 0.4 and 0.6 under the normal, SD growing conditions (e.g. Murfet's L61a) may therefore be a suitable test plant for substances which alter the level of either the promotor or inhibitor or the sensitivity of the plant to a particular balance of these hormones. For this reason an investigation of the effects of several environmental factors which have been suggested to alter the balance of flowering hormones in peas was carried out on L61a.

A large number of plant growth substances and related compounds have been surveyed (table 6.1) using L61a as the bioassay. Several of these substances, including SKF7997, GA_3

and Ethrel, have previously been reported by Moore and Anderson (1966), Barber et al.(1958) and Reid and Murfet (1974b) respectively, to cause significant alterations in the flowering node of peas. However, it is not clear whether their action is a direct effect on the flowering process or due to some indirect effect (e.g. altered vegetative growth). It was hoped that by using L61a, the only substances which could significantly alter the penetrance would be those having direct effects on the flowering processes while the indirect effects would be observed as alterations in the flowering node of the early and, possibly, the late classes.

The endogenous differences between ED and L types were examined by treating L61a with extracts from the two commercial cultivars, Massey (ED) and Greenfeast (L).

MATERIALS AND METHODS

Many separate experiments were carried out and the result are contained in table 6.2. The cutoff between penetrant and impenetrant plants was normally between nodes 17 and 18. However, in some experiments this cutoff was raised by 1 node if a clear zero point occurred at node 19. This change would not alter the penetrance substantially since normally only the classification of 1 plant would be altered. A full list of the flowering nodes obtained in a typical experiment (experiment 2) is given in table 6.3. The number of plants used in each treatment varied from 32 to 60, the number of plants surviving in each treatment being shown in table 6.2. The plants in experiments 1,2 (except for the continuous light treatment), 3, 4, 5, 7, 8, 11 and 12 were planted 2-3 cm below the surface of the growth medium. All plants received an 8 h photoperiod on the trucks unless otherwise stated. Plants receiving vernalisation received 34 days treatment at between 2 and 4^{o}C immediately

after planting. Decotyledonisation was done after 5 days growth in unvernalised plants and at the completion of vernalisation in vernalised plants. Continuous light was given from the start of germination by germinating the plants in Petri dishes on wet cotton wool exposed to 1600 lux from a mixed incandescent-fluorescent source and then transplanting them to the top of the growth medium in the cans. Treatment of plants with the chemicals Ethrel, ABA, GA_3, AMO1618, CCC, androsterone, cholesterol, estradiol and progesterone was done by placing a 10 µl drop of ethanol containing the required amount of each chemical on the dry testa of the seeds before planting. Untreated plants received only ethanol. Heavy seeds weighed 0.31g on average and light seeds 0.16g. Seeds were selected from a single harvesting. The plants treated with aquasol in experiment 12 received this nutrient once a week while the plants treated with Hoagland's nutrient received it twice a week. In experiments 6 and 9 the seeds were germinated in Petri dishes containing 25 ml of either water (controls) or an aqueous solution of the required concentration of SK&F7997 or kinetin and then transplanted to cans, the cotyledons resting on the surface of the growing medium. Table 6.1 contains a list of some properties and synonyms of the chemicals used.

In experiment 10 extracts from Massey (ED) and Greenfeast (L) plants were prepared in order to examine the difference between ED and L types (probable genotypes of *lf E/e sn hr* and *Lf e Sn hr* respectively (Murfet, 1976)). This was done by blending 200 eleven day old plants of each cultivar with 150 ml of methanol. Only the shoot and cotyledons were included. Prior to extraction the plants were raised on the trucks in an 8h photoperiod. After blending, the

methanol was removed at 30°C and the remaining solution centrifuged, filtered and made up to 150 ml with water. L61a seeds were allowed to imbibe this solution for 36 h, by placing the seeds on cotton wool soaked in the extract, and then planted 2 cm below the surface of the growing medium in an 8 h photoperiod at 12.5°C. The plants were transferred to an 8h photoperiod on the trucks after flowering in the EI region was completed.

The L61a seed used in any particular experiment always came from a single harvesting of a group of either F_8, F_9, F_{10} or F_{11} plants. In order to establish whether there was significant genetic variation for penetrance within this seed, seed from impenetrant and penetrant F_8 plants was kept separate and a sample of each progeny grown under an 8 h photoperiod. Although not statistically significant, a small difference in the penetrance was evident between the 2 groups of progeny (table 6.2, experiment 1) and consequently single plant selection will be continued until the F_{12} from which the final L61a will be selected.

RESULTS

Effect of photoperiod, vernalisation and cotyledon removal.

As previously reported by Murfet (1973b) cotyledon removal significantly increases the penetrance of L61a, possibly due to a higher promotor to inhibitor ratio being produced by the cotyledons than the shoot (table 6.2, experiment 1). The cotyledons of other late lines (e.g. L53) probably also produce more promotory levels of the flowering hormones than the young shoots but this difference is larger in L61a due to the presence of penetrance modifiers. Decotyledonisation also leads to a drop in the flowering node of 2.8 nodes in the late region. This drop is probably largely

an indirect effect due to the reduced growth rate of this treatment when compared to intact plants and has been discussed by Murfet (1973b), Reid and Murfet (1974a) and in chapter 5.

Both vernalisation and continuous light lower the penetrance of L61a to zero (table 6.2, experiments 1 and 2). Both treatments have previously been shown to increase the ratio of promotor to inhibitor (chapters 3 and 4 respectively) and this illustrates that treatments which cause a direct alteration of this ratio will cause large alterations in the penetrance of L61a. Both treatments also resulted in a significant (at the 0.001 level) promotion of the flowering node within the early class which would also be expected to occur if the ratio of promotor to inhibitor was raised. Vernalisation of decotyledonised plants (experiment 1) resulted in a 6.8 node (significant at the 0.001 level) reduction of the flowering node supporting the evidence presented in chapter 4 which showed that vernalisation has an effect in the shoot (apex or embryonic leaves) as well as in the cotyledons. No penetrance value for these plants is indicated in table 6.2 since the flowering nodes vary from 15 to 19 and consequently fall into both the EI and L regions. However, it is suggested the plants may be considered as very early flowering representatives of the penetrant class since flowering in the impenetrant region (nodes 10 to 17) is strongly influenced by processes occurring in the cotyledons (e.g. the effect of vernalisation on the stocks in chapter 4) which cannot be occurring in this case.

These results illustrate that the penetrance is easily altered by environmental factors which affect the balance of the flowering hormones and this is an essential requirement in any bioassay for substances which directly affect flowering in peas.

Effect of nutrient types and seed weight.

Neither alteration of the nutrient type (combined with an altered frequency of application) nor the initial seed weight significantly altered the penetrance (table 6.2, experiments 12 and 11). However, the altered nutrient did lower the flowering node by 1 node in the early region (significant at the 0.01 level). Although seed weight has no significant effect the plants from heavy seeds flowered later in both the early and late regions. The rate of leaf expansion was also faster (significant at the 0.001 level) in plants from heavy seeds. These results illustrate that although the rate of vegetative growth and even the flowering node in the early region can be significantly altered by the nutrient available to the plant the penetrance is not significantly altered. This is important in a bioassay system which is to be used to try and isolate substances which mimic the flowering hormones since it may allow the exclusion of substances which exert an effect on flowering through an alteration in the rate of vegetative growth.

Effect of various chemicals.

The only chemical to significantly alter the penetrance of L61a was Ethrel (at the 0.001 level in experiment 2 and the 0.01 level in experiment 3). It also delayed the flowering node in both the early and late regions in experiment 3 (significant at the 0.001 level in the late region but not significant in the early region)(table 6.2). This would suggest Ethrel can cause a significant alteration in either the balance of the flowering hormones or in the sensitivity of the plant to a particular balance. The effect of Ethrel on the flowering of other lines of peas and under different environmental conditions has been examined in chapter 7. Ethrel significantly

(at the 0.001 level) reduced the internode length even in low doses but did not significantly alter the rate of leaf expansion, suggesting it does not act by altering the rate of growth of the plants.

Several chemicals had no significant effect on the penetrance or the flowering node in either the early or late regions at the concentrations used. These included the steroids, androsterone, cholesterol, estradiol and progesterone , and the reputed inhibitor of gibberellin synthesis, CCC. Further, extracts from Massey and Greenfeast plants did not significantly alter either the penetrance or the flowering node in the early or late regions. The fact that no response was observed in the above experiments could be due firstly to a true null response to these treatments in peas, secondly to insufficient chemical reaching a site where it can exert an effect due to the limitation of the application technique or thirdly, to the substance being metabolised by the plant so rapidly that a single dose is ineffective. It is worth noting that the rate of leaf expansion and internode length were not affected by any of these chemicals and this may lend support to the second and third alternatives.

ABA and kinetin significantly lowered the flowering node in the early region (experiments 4, 5 and 6). Promotion by ABA has also been observed in L60 where a 1 node promotion of the flowering node (significant at the 0.001 level) was observed with a 20 µg treatment under SD conditions. ABA also reduces the length between nodes 1 and 6 and the rate of leaf expansion (the effect of ABA on leaf expansion was not recorded in the present experiments but has been recorded in several other experiments). It is therefore suggested that ABA affects

the flowering response indirectly by reducing the vegetative vigour of the plant resulting in flowering at a lower node even though the time of initiation may not be varied. A similar explanation would also explain the promotion (significant at the 0.01 level) of flowering in the late region by ABA which was observed in experiment 5. Kinetin did not cause any significant change in the length between nodes 1 and 6 or the number of expanded leaves and may have its effect due to some interaction between the levels of the flowering hormones and the level of cytokinins.

At the dose rates used in the present experiments (table 6.2, experiment 7) neither GA_3 nor AMO1618 (an inhibitor of gibberellin synthesis) significantly altered the penetrance of L61a or the flowering node in the early region. However, in the late region GA_3 significantly delayed the flowering node (at the 0.05 level) while AMO1618 significantly promoted it (at the 0.001 level). Coinciding with these differences were increased internode lengths and rate of leaf expansion after treatment with GA_3 and decreased internode lengths and rate of leaf expansion after treatment with AMO1618. These results do not indicate a direct alteration of the promotor to inhibitor ratio or of the sensitivity of the plant to a particular ratio of the flowering hormones by GA_3 or AMO1618.

DISCUSSION

The most commonly used types of bioassay for substances which affect flowering is to apply the substances to the imbibing seeds (e.g. Highkin, 1956; Moore and Bonde, 1962; Tomita 1964), the leaves (Evans, 1966; Biswas et al.,1967), the apex (Evans, 1966) or the growing medium (Cleland, 1974; Jacobs and Suthers, 1971). All of these systems are primarily

designed to test for either an inhibition or promotion of the flowering process although both may show up under some circumstances. Often it is not indicated whether the test system is able to respond easily to alterations in the levels of the flowering hormone(s). With extractions, entirely different plants are sometimes used as the bioassay system to those from which the extractions were made (e.g. Cleland, 1974; Bonner & Bonner, 1948) and consequently the assumption must be made that the flowering hormones are similar in both species. Although this has been shown to be the case in some closely related species (Lang, 1965), it seems unlikely to be a general rule. The suggested bioassay system using L61a allows for both promotion and inhibition and has been shown to be sensitive to changes in the ratio of promotor to inhibitor brought about by the changes in the photoperiod and temperature. Further, when extracts from peas are made it allows the testing of these substances on the same organism and therefore makes no assumptions as to the between species variation in the control of flowering. Indirect effects on the flowering process (e.g. by altered vegetative growth) have been shown not to significantly influence the penetrance of L61a whereas these effects would be confounded with direct effects in other bioassay systems (Cleland, 1974; Evans, 1966; Murfet and Barber, 1961; Sprent, 1967; Moore and Bonde, 1962). These indirect effects however need to be recorded if a full understanding of the flowering process is to be obtained and, although confounded with direct effects, show up in L61a as changes to the flowering node of the early and, to a lesser extent, the late classes. The use of L61a as a bioassay does, however, have some disadvantages. Large numbers of plants are required to obtain definite answers and

the environment needs to be controlled accurately so that the penetrance of control plants is close to 0.5. Consistent small increases (insignificant with the number of plants used) in penetrance are observed after the application of some chemicals and may result from the development of the cotyledons and shoot becoming slightly out of phase with each other when compared to control plants. Long growth periods are also required before all the answers are obtained even though the penetrance could be determined relatively quickly (within a maximum of three weeks). As with all bioassays some uncertainty about negative results is always left due to the limitation of the application techniques and the amount of substance that should be applied.

The simultaneous application of extracts from different flowering genotypes should allow effects attributable to the normal substances in a plant to be eliminated since the vast majority of compounds would be similar in both genotypes. Most workers have compared the flowering of plants treated with extract with those of control plants only, and have assumed the stimulus should be a promotor (Lincoln et al., 1961; Schwabe, 1969). Consequently inhibiting effects may well have been overlooked. Sometimes (e.g. Cleland, 1974) extracts from both flowering and non-flowering plants have been compared, usually with no differences being observed. This would not be unexpected if both sets of plants were close to the threshold for flowering since very little difference in the absolute amounts of the flowering hormones might be present (e.g. extracts from penetrant and impenetrant L61a plants when approximately 16 nodes are present or from L63 plants induced to flower by 1LD cycle and from those left under SD conditions).

The use of different flowering genotypes under environmental conditions which cause the largest differences between them should allow the maximum difference in the levels of the flowering hormones to be examined. However, in the present experiments no significant difference occurred between plants treated with extracts from Massey and Greenfeast plants. This could be caused by the extraction procedure having destroyed the hormones or resulted in the ratio of inhibitor to promotor being similar in both extracts for some reason (e.g. degradative enzymes being released which could deactivate one or other hormone) or for similar reasons to those given for chemicals which had no effect. The list of possible explanations is long but the general system may allow useful analysis of the differences between the different flowering genotypes in future.

The results indicate that of the chemicals analysed by the L61a bioassay, Ethrel, gibberellic acid and AMO1618 are the three chemicals which warrant the most attention and further work with these chemicals is contained in chapters 7 and 8. Ethrel appears to have a direct, inhibitory influence on the flowering process. Whether this occurs because Ethrel increases the effective level of inhibitor, reduces the effective level of promotor or alters the ratio of inhibitor to promotor required for flowering is not indicated. GA_3 delays flowering in the late region and AMO1618 promotes it but whether the altered rate of vegetative growth is responsible is not known. It is possible that GA_3 is delaying the effect of age on the *Sn* gene and therefore to some extent mimics the action of the *Hr* gene. This suggestion is supported by the fact that the flowering node of impenetrant L61a plants is not significantly altered.

Table 6.1 This table contains a list of the chemicals tested for their effect on flowering using L61a as the bioassay, the abbreviations used in the text, the reported effect of the chemicals in the literature and references to them.

NAME	ABREVIATION USED (IF ANY)	REPORTED ACTION	REFERENCES
Abscisic acid	ABA	Plant hormone. Inhibitor of active growth.	Addicott & Lyon (1969)
Kinetin	-	Growth regulator. Member of the cytokinin group.	Letham (1967)
Gibberellic acid	GA_3	Plant hormone. Member of the gibberellin group.	Jones (1973)
Ethrel, solution of 2-chloroethylphosphonic acid	-	Breaks down in plants to release ethylene.	Warner & Leopold (1969)
2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride	AMO1618	Growth retardant. Acts by inhibiting gibberellin synthesis.	McComb & McComb (1970) Cathey (1964)
2-chloroethyletrimethyl-ammonium chloride	CCC	Growth retardant, acts by inhibiting gibberellin synthesis.	Cathey (1964)
Tris-(2-dimethylamine-ethyl)-phosphate trihydrochloride	SK & F7997	Inhibitor of steroid synthesis.	Moore & Anderson (1966)
Androsterone	-	A steroid and acts as a hormone in mammals.	White, Handler & Smith (1967)
Estradiol	-	A steroid and acts as a hormone in mammals.	White, Handler & Smith (1967)
Progesterone	-	A steroid and acts as a hormone in mammals.	White, Handler & Smith (1967)
Cholesterol	-	A steroid found extensively in animals.	White, Handler & Smith (1967)

Table 6.2 The penetrance, mean node of first initiated flower ± S.E. for plants flowering in the early (nodes 10-17) and late regions (nodes 18-34), the length ± S.E. and number of leaves expanded ± S.E. for L61a *(If e Sn hr)* plants given the various environmental and chemical treatments indicated. Control plants were given an 8 h photoperiod on the trucks, these conditions applying to all other treatments apart from the variable indicated. Vernalisation (V) and continuous light (LD) were given from the start of germination while decotyledonisation (decot) was performed on days 5 and 6. The nodes between which the length measurements were taken and the time at which the number of leaves expanded was recorded varied from experiment to experiment, but provide useful evidence as to the relative vigour of the various treatments within a single experiment.

Experiment	Treatment	Penetrance	Flowering Node				Length	Leaves expanded
			Early		Late			
			$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.
1	EI parents	.68	14.81 ± .44	16	28.03 ± .26	30	14.10 ± .17	
1	L parents	.80	15.00 ± .50	9	27.97 ± .39	38	13.33 ± .16	
1	intact, UV							
1	intact, V	0	12.33 ± .11	46	-	0	14.45 ± .17	
1	decot, UV	1.00	-	0	24.19 ± .23	43	5.31 ± .16	
1	decot, V	-	17.39 ± .14	46	-	0	4.38 ± .08	
2	control	.25	13.38 ± .30	24	26.13 ± .81	8		
2	L53	1.00	-	0	26.50 ± .33	16		
2	LD	0	11.67 ± .14	30	-	0		
2	V	0	11.77 ± .11	30	-	0		
2	decot	1.00	-	0	21.03 ± .20	29		
2	Ethrel	.81	15.60 ± .68	5	25.95 ± .61	22		
3	control	.60	14.25 ± .58	12	25.33 ± .23	18	8.42 ± .14	13.87 ± .16
3	Ethrel .01 mg	.70	14.91 ± .37	11	25.95 ± .32	19	7.38 ± .14	13.90 ± .15
3	Ethrel .1 mg	.83	15.17 ± .40	6	26.33 ± .24	24	6.50 ± .13	13.90 ± .15
3	Ethrel .48 mg	.90	15.33 ± .33	3	27.04 ± .31	26	5.97 ± .13	14.03 ± .13
4	control	.56	14.50 ± .14	14	29.11 ± .36	18	4.30 ± .09	
4	ABA 10 µg	.48	12.57 ± .45	14	28.85 ± .81	13	3.95 ± .12	

Table 6.2 (Continued)

Experiment	Treatment	Penetrance	Flowering node: Early $\bar{x} \pm$ S.E.	Late n	Late $\bar{x} \pm$ S.E.	Length n	Length $\bar{x} \pm$ S.E.	Leaves expanded $\bar{x} \pm$ S.E.
5	control	.72	14.69 ± .30	16	28.93 ± .35	42	3.83 ± .05	
5	ABA 20 ug	.66	13.30 ± .44	10	27.00 ± .67	20	3.24 ± .16	
6	control	.33	13.10 ± .28	20	22.50 ± .37	10	4.50 ± .13	24.00 ± .30
6	Kinetin 20 ppm	.52	12.27 ± .38	11	22.92 ± .40	12	4.67 ± .18	23.78 ± .45
6	Kinetin 100 ppm	.44	11.93 ± .20	14	22.64 ± .58	11	4.55 ± .12	24.04 ± .42
7	control	.25	11.78 ± .26	27	25.33 ± .37	9	7.20 ± .10	9.89 ± .09
7	GA 10 ug	.37	12.19 ± .27	22	26.54 ± .40	13	29.83 ± .79	11.17 ± .13
7	AMO 100 µg	.44	11.83 ± .22	18	23.33 ± .26	14	4.62 ± .08	9.45 ± .10
7	CCC 100 µg	.17	12.07 ± .18	29	24.17 ± .79	6	6.67 ± .11	9.77 ± .08
7	CCC 500 µg	.29	12.21 ± .29	24	25.70 ± .40	10	6.41 ± .10	9.91 ± .09
8	control	.53	12.60 ± .40	15	26.65 ± .31	17	7.57 ± .10	20.84 ± .13
8	Androsterone 1 mg	.70	12.44 ± .18	9	25.32 ± .32	21	7.28 ± .20	21.14 ± .37
8	Cholesterol 1 mg	.70	12.67 ± .37	9	25.76 ± .30	21	7.02 ± .18	21.00 ± .31
8	Estradiol 1 mg	.56	12.21 ± .30	14	26.13 ± .49	16	7.33 ± .15	20.78 ± .37
8	Progesterone 1 mg	.75	12.80 ± .80	5	26.07 ± .26	15	7.20 ± .12	21.19 ± .46
9	control	.87	12.67 ± .21	6	26.34 ± .32	40	5.23 ± .07	22.46 ± .23
9	SKF7997 1000 ppm	1.00		0	25.39 ± .47	20	4.17 ± .14	21.25 ± .33
10	Massey Extract	.89	15.17 ± .48	6	23.14 ± .23	50		
10	Greenfeast Extract	.82	15.40 ± .27	10	22.96 ± .29	45		
11	light seed	.53	13.64 ± .47	11	25.79 ± .42	19	3.70 ± .11	7.73 ± .10
11	heavey seed	.50	14.57 ± .48	14	27.21 ± .66	14	3.73 ± .10	8.64 ± .10
12	Aquasol	.29	13.55 ± .26	22	27.22 ± .88	9	8.05 ± .23	8.09 ± .13
12	Hoaglands	.31	12.55 ± .25	20	28.11 ± .26	9	8.35 ± .11	7.96 ± .20

Table 6.3 Distribution of the node of first initiated flower for L61a plants exposed to an 8 h photoperiod and either given 34 days of vernalisation (V), decotyledonised on day 5 (decot), treated with 480 µg of Ethrel on the dry testa or left untreated (control). A sample of L53 was also grown in an 8 h photoperiod as well as a group of L61a plants exposed to continuous light from the start of germination. The dividing line between penetrant and impenetrant plants was taken as lying between nodes 17 and 18.

Treatment	Node of first flower																						Penetrance
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30 – 31		
Control	1	0	6	7	4	4	2	-	-	-	-	1	0	0	0	1	1	3	2	-	-	-	.25
L53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	4	3	5	-	-	-	1.00
LD	1	12	13	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
V	-	9	20	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
decot	-	-	-	-	-	-	-	-	-	2	7	11	6	3	-	-	-	-	-	-	-	-	1.00
Ethrel	-	-	-	1	0	0	3	1	-	-	-	2	0	3	2	2	4	3	2	1	1	2	.81

CHAPTER 7

ETHYLENE

Effects of Ethrel and Indole -3- Acetic Acid on the Flowering of L58 *(lf o sn hr)*.

INTRODUCTION

In the previous chapter the ethylene releasing compound Ethrel was shown to be able to significantly increase the pentrance of L61a. The first indication that ethylene may be able to affect flowering came when L58 plants that had been germinated on wet cotton wool in a Petri dish flowered at a later node than those germinated in the usual manner 3cm deep in a vermiculite-gravel mixture (chapter 3). During germination a smell of organic gases built up within the Petri dish and the radicles of the seedlings tended to thicken and curl, appearing similar to the ethylene-treated plants described by Lieberman and Kunishi (1972). It was decided therefore to examine the effect of the ethylene-releasing compound Ethrel (Cooke and Randall, 1968 Warner and Leopold, 1969), on the flowering of L58. Since high concentrations of auxin have been reported to increase ethylene production (Zimmerman and Wilcoxon, 1935; Morgan and Hall, 1962; Burg and Burg, 1966a) the effect of different concentrations of indole-3-acetic acid (IAA) was also studied. Previous workers have reported that exogenously supplied auxins either delay (Leopold and Guernsey, 1953) or have no effect on (Haupt, 1952) the flowering node of early pea varieties.

MATERIALS AND METHODS

L58 was used throughout this work. The data in fig. 7.1 and the left half of table 7.1 come from experiment 1, in which 10 treatments were used with 18 plants per treatment. The dry seeds of the controls were treated on the testa with 10 µl of ethanol

and planted 3cm deep in pots, the shoots emerging about day 7. The effect of IAA was examined by applying as a single dose 1 mg of IAA in 10 µl of ethanol to the testa of the dry seed. After the ethanol had evaporated the seeds were set to germinate 3cm deep in the vermiculite-dolerite mixture in the same manner as the controls. The Petri dish effect was checked by germinating the seed on wet cotton wool in both open and closed Petri dishes. Finally, a range of Ethrel concentrations was tested by germinating the seed in open Petri dishes on cotton wool soaked in an aqueous solution containing 1,2,5,10,20 or 40 p.p.m. of Ethrel, which is a solution of 480g 2-chloroethylphosphonic acid per litre of formulation. Seedlings were transferred from the Petri dishes to the pots on days 3 and 4 in such a way that the cotyledons remainced exposed. Continuous light was given by extending the natural summer photoperiod to 24h using light from a mixed fluorescent-incandescent source.with an intensity of 2200 lux.

Experiment 2 (right half of table 7.1) examined the effect of lower concentrations of IAA (5, 50 and 500 µg per seed). The method and conditions were the same as in experiment 1 except that the supplementary lighting was of a lower intensity (900 lux).

In both experiments the temperature was variable but did not drop below 15^{o}C.

RESULTS AND DISCUSSION

The results in table 7.1 illustrate that germination in closed or open Petri dishes delays flowering by 1.1 and 0.8 nodes respectively when compared with the control plants germinated 3cm below the surface of a vermiculite-gravel mixture.

The delays are significant at the 0.01 and 0.05 levels respectively. From fig. 7.1 it is clear that even the lowest concentration of Ethrel (1 p.p.m.) has significantly (0.001 level) delayed flowering over and above any Petri dish effect. The delay levels off at a concentration of 10 p.p.m., the maximum delay being 3.7 nodes. This effect is repeatable, delays of between 3.4 and 4.3 nodes having been recorded in three further experiments with Ethrel using higher concentrations, and either the present or different application techniques. Ethrel also induced concomitant effects on stem thickness, internode length etc., characteristic of ethylene treatment, and it seems reasonable to attribute the flowering delay to ethylene produced from the breakdown of 2-chloroethylphosphonic acid.

IAA had no significant effect on the flowering node of L58 until the high dose of 1 mg per seed was used. This resulted in a 1.7-node delay (significant at the 0.001 level), the plants also having short thick epicotyls and reduced hypocotyl extension. Since high doses of auxin have been shown to increase ethylene production, and the plants treated with IAA appeared similar to those treated with low concentrations of Ethrel, it is possible that IAA has a secondary effect on flowering in peas through the stimulation of increased ethylene production. Previously, ethylene has been reported to influence floral initiation in only a few sepcies, e.g. the promotion of flowering in pineapples (Rodriguez, 1932; Lewcock, 1937; Cooper and Reese, 1941) and its inhibition in *Xanthium pennsylvanicum* (Abeles, 1967). In both species auxin has a similar effect to that of ethylene (Bonner and Thurlow, 1949; Burg and Burg, 1966b). The contrasting response to ethylene in different species once again shows that generalisations cannot be made about the effect of various plant

growth regulators on whole plants, even though similar biochemical roles may be involved.

The results do not show the relationship between the delaying effect of ethylene and the endogenous control of flowering in peas. However, certain parallelisms can be seen between the response to Ethrel and the action of the gene *Sn*, which controls the production of a graft-transmissible flower inhibitor (Paton and Barber, 1955; Murfet and Reid, 1973). L58 scions grafted onto stocks (with cotyledons) of genotype *lf e Sn hr* are delayed to approximately the same node as L58 plants treated on the cotyledons with those concentrations of Ethrel giving the largest delay. The *Sn* gene also reduces internode length, delays the transition from two to more than two leaflets per leaf (Barber 1959) and opposes reproductive development after floral initiation has occurred (Murfet, 1971a). In the present experiment, 40 p.p.m. of Ethrel reduced the length between nodes 1 and 6 by 43% (significant at the 0.001 level). Continued application of Ethrel resulted in the first pod being set an average of 2.6 nodes after initiation had occurred. In the controls this difference was 0.1 of a node (the difference between these two results is significant at the 0.001 level). The activity of the gene *Sn* appears to be regulated by the length of the photoperiod, little, if any activity being observed in continuous light. The production of ethylene in peas has been shown to be influenced by light (Goeschl et al., 1967) and there have been instances in which the interruption of the long dark period in short-day plants by a flash of red light has led to a decrease in ethylene production (Galston and Davies, 1970). These results combined with the effects of Ethrel on the penetrance of L61a (chapter 6) suggest the need for further

investigation of the relationship between ethylene and the gene *Sn*. This has been attempted in the following section by using other genotypes and techniques which allow the endogenous levels of ethylene to be examined.

Effect of Ethrel on Several Lines of Peas and the Relationship Between Endogenous Ethylene, the *Sn* Gene and Flowering.

INTRODUCTION

In order to obtain an insight into the interaction of ethylene with some of the genes which control the change from vegetative to reproductive growth the effect of Ethrel on the lines 53, 59, 64, 60, 53, 51y, 68, 63 and 7 was examined using several sets of environmental conditions. The genotypes of these lines vary considerably at the 4 major loci, *lf*, *e*, *sn* and *hr*, which control flowering (Murfet, 1971a, 1971b, 1973a, 1975) (see table 2.1 for details). However, it should be noted that considerable variation exists between these lines at other major as well as minor loci and consequently caution must be used when interpreting differing responses as due to the differences at the four loci specified. The use of two lines (L58 and L68) of the genotype *lf e sn hr* allows some insight into the importance of this problem.

Since Ethrel has been shown to mimic several actions of the gene *Sn* the endogenous levels of ethylene in two closely related lines (L53 and L58) which differ at the *Sn* locus (Murfet, 1971a) were studied under an 8h photoperiod to determine if the *Sn* gene had any direct observable effect on the quantity of ethylene given off by the plants. It was hoped this evidence would clarify the relationship between ethylene and the gene *Sn*.

MATERIALS AND METHODS

The three lines, 58, 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)*, used in experiment 1 (table 7.2) were exposed to continuous light from the start of germination by placing 4 seeds in a Petri dish on wet cotton wool which was soaked with 35ml of either water or an aqueous solution containing 2, 20 or 100 ppm of Ethrel. They were transplanted to the surface of the growing medium on day 3 or 4, the radicles being 2-4cm long at this time. No plants of the L63, 2ppm treatment survived to a stage where flowering could be scored on the main shoot. The main reason for this failure was that the apex died prior to flowering, a characteristic prevalent in all L63 treatments in this experiment.

In experiment 2 (table 7.3) seeds of lines 58, 59, 64, 60, 53 and 51y were treated on the dry testa with 10 µl of ethanol containing either no Ethrel or 24, 96 or 480 µg of Ethrel. After the ethanol evaporated the seeds were planted 2cm beneath the surface of the growing medium under an 8h photoperiod. If the shoots did not come through the surface by about day 10 the plants were dug-up and the shoot exposed to the light. This was necessary since the Ethrel caused the plants to become ageotropic. Plants treated with Ethrel also produced a large number of laterals which were regularly removed. Plants given continuous treatment were treated with 480 µg of Ethrel at the commencement of the experiment and then watered with 100ml of an aqueous solution containing 100 ppm of Ethrel once a week for 5 weeks, starting on day 15.

In experiment 3 (table 7.4) similar techniques were used as in experiment 2 except that continuous light was used instead of an 8h photoperiod, only one concentration of Ethrel

was used (480 µg) and only three lines, L58, L59 and L68 were tested.

In experiment 4 a group of 24 L64 plants were treated with 480 µg of Ethrel as described in experiment 2. The flowering node of these plants was recorded and 5 of their progeny (less if a plant had a smaller yield) were treated in the same way and then the regression of mean progeny flowering node against parental flowering node obtained. The results are contained in fig. 7.2.

In experiment 5 (table 7.5) L7 plants were grown under an 8h photoperiod and either decotyledonised after 16h imbibition and grown on White's nutrient agar until leaf 4 was expanded and then transferred to cans, decotyledonised on day 5, left intact, or treated on the dry testa with 480 µg of Ethrel and treated as in experiment 2.

In experiment 6, L63 plants were grown under an 8h photoperiod until 6 weeks old (approximately 25 leaves expanded). The plants were then exposed to 1 LD cycle (32h of light) and treated with either 10 µl of ethanol on the fourth expanded leaf back from the apex 8h after the start of the LD cycle (4pm) or in the same way with the 10 µl of ethanol containing 480 µg of Ethrel at 4pm on either the first or second days before the LD cycle, the day of the LD cycle or the first, second or third days after the LD cycle. A further group received 480 µg of Ethrel on each of these 6 days at 4pm. The plants were transferred to a long photoperiod after approximately 40 leaves had expanded. The results are contained in table 7.6.

In experiment 7, ethylene production was measured in L58 *(lf e sn hr)* and L53 *(lf e Sn hr)* plants grown under an 8h photoperiod (SD). Also the production of ethylene by L53 was compared under continuous light (LD) and an 8h photoperiod. Groups of 4 seeds were germinated in pure vermiculite and then transferred at the time the shoots came through the surface (approximately day 7) to 300ml wide-necked reagent bottles containing vermiculite. The bottles were then sealed with rubber stoppers which were fitted with glass tubing over which sealed surgical rubber tubing was placed. A 1 ml sample of gas could then be taken from the bottles after a certain period by pushing the needle of a syringe through the rubber tubing into the bottle. Each bottle was sampled twice, the ethylene content of the gas being measured with a Pye series 104 gas chromatograph. The column was packed with Porapack Q and heated to 50°C with a flow rate of 40 ml/min. Three separate runs were made, the results being contained in table 7.7. In the first run the treatments L58SD, L53LD and 2 bottles of L53SD were tested after 120h incubation. A flask containing 100ml of a 100 ppm solution of Ethrel in 0.001 N KOH was included to show the retention time of ethylene under the conditions used. Warner and Leopold (1969) have previously shown Ethrel is nearly completely degraded in dilute alkali to release ethylene and phosphate and chloride ions. In the second run 2 bottles of both L58SD and L53SD were examined whilst in the third run 2 bottles containing only vermiculite and 1 bottle each of L58SD and L53SD were examined. Samples of gas were taken after 72h incubation in the second and third runs. Also flasks containing 2 and 10 ppm of Ethrel in 100ml of 0.001 N KOH were tested to allow some approximation of the actual quantities of ethylene produced by the plants. This was based on the assumption that

Ethrel is completely broken down to release 1 molecule of ethylene per molecule of Ethrel as suggested by Warner and Leopold (1969). In each run the weight of the 4 dry seeds used in each bottle was similar. To compare the relative amounts of ethylene produced by the different treatments within a particular run the height of the peak was considered to be sufficient. However, the areas under the peaks were determined in the third run to allow some estimate of the quantity of ethylene given off by the plants. For comparisons it was felt that height was a more accurate measurement due to the poor integration techniques available.

RESULTS

The results in tables 7.2, 7.3, 7.4 and 7.5 show that Ethrel is capable of increasing the flowering node of lines 58, 59, 64, 60, 53, 51y and 7 under an 8h photoperiod and of lines 58, 53, 63, 68 and 59 under continuous light (all delays significant at the 0.001 level when 480 µg of Ethrel was used.) However, although Ethrel is general in its effect, the size of the delay varies considerably from one line to another even within one experiment. For example the two closely related lines, L58 *(lf e sn hr)* and L59 *(lf E sn hr)* in table 7.3 differ significantly in the extent they are delayed by differing concentrations of Ethrel (at the 0.001 level using the interaction term from an analysis of variance on the L58 and L59 data in table 7.3). For example a delay of 5.19 nodes occurs in L58 and of only 2.63 nodes in L59 when 480 µg of Ethrel was used. This difference in response does not appear to be due to any promotory effect of the gene *E* in L59 since from table 7.4 the size of the delay in L59 is seen to be intermediate in size to the delays in L68 and L58

although both L58 and L68 are reported to possess the same genotype *(lf e sn hr)* at the major loci controlling flowering (Murfet, 1971a, 1973a). As well as having varying effects on the size of the flowering delay it is interesting to note that a particular concentration of Ethrel also had differing effects on the vegetative growth of the different lines. This is illustrated by the fact that the length between nodes 1 and 6 was consistently reduced by the greatest percentage in L58 (tables 7.3 and 7.4). However, this measurement does not appear to tell the whole story, plants of L58 and L53 being very "sick" in appearance when treated with 480 µg of Ethrel even after 4 to 5 weeks growth, while L51y and L68 appear almost unaffected by this treatment at this time. Lines 60, 59 and 64 are somewhat intermediate in their response between these two groups. Whether this differing vegetative response to Ethrel is responsible for the differing flowering responses (e.g. between L58 and L68) is being examined.

When exposed to continuous light from the start of germination, untreated L53 and L63 plants flowered later than the untreated L58 plants (significant at the 0.05 level)(table 7.2). This difference could either be due to reasons outlined previously (page 39) or to a higher sensitivity of lines 53 and 63 to the ethylene which appears to be produced within the untreated Petri dishes. An analysis of variance on the flowering nodes for L58 and L53 plants (L63 was not included due to the failure of the L63, 2 ppm treatment) showed that the interaction between Ethrel treatment and genotype was significant at the 0.05 level. This would seem to come about due to a larger response by L53 to the lowest concentration of Ethrel (2 ppm) and would support the suggestion that L53 may be more sensitive to ethylene than L58

(at least with regard to the flowering node). The nodes to which lines 53, 58 and 63 are delayed by the highest concentration of Ethrel used (100 ppm) were not significantly different.

Apart from the untreated plants, the means for L64 plants in experiment 2 (table 7.3) have large standard deviations, the flowering nodes varying from 10 to 16 with only one plant out of 43 flowering from node 14. This variation was examined to see whether it was heritable by treating a group of L64 plants with 480 µg of Ethrel under an 8h photoperiod, scoring the flowering nodes and then growing the progeny under similar conditions. A regression of the mean flowering node of the offspring on the parental flowering node gave a significant slope (at the 0.05 level) indicating a small heritable component (fig. 7.2). However, the large variation continued to occur even within the progeny from one plant. It is suggested that a form of impenetrance is occurring in which Ethrel either lowers the ratio of promotor to inhibitor reaching the apex to a level close to the threshold for flowering or lowers the threshold itself. Plants will then either flower in the early region (nodes 10-13) when the cotyledons are the major source of the flowering hormones or not until the ratio coming from the shoot becomes promotory (nodes 15-18). This occurs because the cotyledons of L64 under an 8h photoperiod produce a more promotory balance of the flowering hormones than does the young shoot (Reid and Murfet, 1975a). A very small proportion of untreated plants of this genotype have also been reported to flower above node 15 (Murfet, 1973a) presumably for the same reason as given above. Although the range of the possible flowering nodes is smaller this situation is analogous to that observed in intact L61a plants under an 8h photoperiod (see chapter 7). It might be suspected that

the flowering node of L60 *(lf E Sn hr)* would also become bimodal upon treatment with Ethrel under an 8h photoperiod, but this was not observed. Presumably either the balance of the flowering hormones is further from the threshold in this line than in L64 or it is less sensitive to applied Ethrel.

The data in table 7.5 indicate that the flowering node of L7 *(lf^a E Sn hr)* is not determined (at least in all plants) before germination since treatment with 480 µg of Ethrel and decotyledonisation after 13h inhibition were both able to significantly delay the flowering node (at the 0.001 level). The number of nodes laid down in the apex after 24 hours inhibition was 6.11^{+}_{-}. 11 (from a sample of 9 plants) indicating that an alteration in the flowering node is possible until very close to the time of initiation. Decotyledonisation on day 5 resulted in no significant alteration of the flowering node presumably because the plants had already initiated. Plants dissected on the 5th day possessed 8.13^{+}_{-} .13 nodes (sample of 8 plants). It should however be noted that in no case could the typical "bulge" of a flower primordium be seen in the leaf axil during these dissections suggesting axillary bud development is lagging substantially behind the development of leaf primordia in this particular line. This would seem different to the ED and L lines dissected where the flower bud at a particular node is normally observable by the time the leaf primordium is initiated. It raises the possibility that the nature of the axillary bud (either vegetative or floral) may not be determined until after the leaf primordium has been initiated in L7 although on the present evidence the determination would be made before day 5.

Treatment of L63 *(lf e Sn Hr)* with 480 µg of Ethrel

does not appear to be able to reduce the ability of 1LD cycle to induce flowering (table 7.6). This could be due to the method of application, the Ethrel not reaching a site where it can exert an effect. However, in the plants treated with Ethrel for the six days surrounding the 1 LD cycle typical symptoms of ethylene treatment were observed and premature senescence was induced in 9 plants, death occurring before a visible flower bud had been formed. Even in the plants surviving this treatment the ability to respond to 1 LD does not appear to have been altered. This would suggest that the Ethrel was penetrating the plant at least to a site where it could cause a physiological response, but that it is unable to directly affect the sensitivity of L63 plants to a LD cycle. The premature senescence observed was restricted to areas of the shoot close to the site of application, including the apex. Whether this senescence was in any way similar to naturally induced senescence which normally follows the production of seeds is unknown but the leaves affected did become chlorotic prior to their death. This action of a massive dose of Ethrel is opposite to the effect of the *Sn* gene which has been reported to delay senescence by delaying the flowering node and by increasing the amount of post reproductive development in SD conditions (Murfet, 1973a).

There appears to be no consistent difference in the ability of L58 and L53 plants under SD to produce ethylene although some differences did occur in the three runs performed (table 7.7). The production of ethylene also does not appear to be affected by photoperiod in L53 although only a single bottle containing four plants was examined under continuous light. It is clearly shown in the third run that the ethylene produced in the flasks is produced predominantly by the plants and not released from

the growing medium. The approximate level of ethylene in the flasks containing plants for the third run was 5.3 ppm of ethylene or 21 nl/g of seeds/h (average for L58 and L53 results) and was 0.6ppm in the flasks without plants.

DISCUSSION

Although Ethrel is capable of significantly delaying the flowering node and reducing the length between nodes 1 and 6 in all genotypes examined under both long and short photoperiods, it is clear that different genotypes respond to differing degrees to the application of Ethrel. The difference in the flowering response does not appear to be associated with the genes *E* and *Hr* but there is some evidence to suggest *Sn* may be responsible to a small extent since L53 plants respond to a larger extent than L58 plants when treated with small doses of Ethrel. Whether the interactions of *Sn* with *E* and *Hr* alter the responses is not clear. The balance of the flowering hormones existing in the plant during the early growth does not appear implicated in the differential response since L58 plants are delayed to a later node than are L60 plants even though L60 cotyledons and shoots have been shown to produce a more inhibitory balance of the flowering hormones than L58 (Murfet, 1971c; Wall et al., 1974). It appears other as yet undetermined genetic systems are responsible for the largest part of these different responses as illustrated by the degree of difference between L68 and L58. How these systems operate is unkown but it could be through a differing ability to destroy or inactivate ethylene, different levels of other hormones or through altered levels of endogenous ethylene.

Little work appears to have been reported on species which show such genetic diversity in their response to applied

growth regulators and hormones. This probably occurs since most physiologists have restricted their studies to a specific cultivar and assume their studies on this cultivar are representative of the species as a whole. Some notable exceptions do occur, for example the effect of GA_3 on flowering in different cultivars of peas (Dalton and Murfet, 1975) and on internode length in varieties of peas and maize (Brian and Hemming 1955; Phinney 1956). The work of Tal and Imber (1970, 1971) and Imber et al. (1970) on wilty tomato mutants show that the level of various hormones are under direct genetic control and may vary over a wide range in different genotypes. In all these examples a clear morphological difference has been observable prior to hormone application, the hormone application usually masking this effect or at least producing a consistent effect within each morphological group. However in the present example there does not appear to be any direct relationship between the size of the response to ethylene and the morphological classification of the line. In this respect it is similar to the different responses shown in peroxidase activity by the pea cultivars Massey and Greenfeast after application of IAA (Mills, unpub.). The occurrence of different responses within one species illustrates how invalid it may be to extrapolate from one species to another as is so often done in the literature. It also highlights the need for multi-disciplinary studies of the control of development in plants, in this case a joint genetical, whole plant physiological and biochemical study being required.

The relationship of the *Sn* gene to endogenous ethylene levels is still far from being fully clarified. However, it would appear that the gene *Sn* does not directly

control the amount of ethylene given off by pea plants, at least within the accuracy of the analytical methods used in the present study. A similar statement can also be made about photoperiod length. The fact that even high, repeated doses of Ethrel could not alter the sensitivity of L63 plants to 1 LD cycle would support these conclusions. The fact that continuous application of Ethrel to both lines 58 and 64 (table 7.3) did not increase the size of the delay over that produced by a single dose might suggest ethylene needs be present at the start of some sequence of events in order to be effective. The later doses of Ethrel could be observed to affect vegetative growth and would certainly have been given (at least in some cases) prior to the time of flower initiation.

The relationship of the other major flowering genes to the endogenous levels of ethylene have not been examined. However, the results of experiments using Ethrel treatment would suggest that *Hr* and *E* do not operate through ethylene metabolism since Ethrel is effective in delaying flowering in both *Sn* and *sn* plants while *Hr* and *E* are only effective (at least to a large extent) in *Sn* plants. The fourth major flowering locus, *lf*, appears to control the sensitivity of the apex to the ratio of inhibitor to promotor reaching the apex (Murfet, 1971c, 1975). The interaction of alleles at this locus with ethylene has not been examined and appears to be required.

It would seem strange if the most potent flower inhibitory compound yet found for early cultivars of peas does not possess some endogenous role in the control of the flowering processes, especially when it has been shown to be produced by

them in fairly high quantities. However, this endogenous role is not revealed by this study, although several alternatives have been excluded. Possibly ethylene is only a part of a general system of controlling hormones, the effect of observing or analysing for any one always leading along a hopeful path to a dead end beyond which further insight into the "black box" cannot be made without simultaneous measurements of many other compounds within the plant.

Table 7.1 The mean node of first initiated flower in L58 *(lf e sn hr)* plants germinated either in vermiculite (controls), on cotton wool in open or closed Petri dishes, or in vermiculite and treated with IAA. The plants were exposed to continuous light.

Experiment 1			Experiment 2		
Treatment	$\bar{x}$ ± S.E.	n	Treatment	$\bar{x}$ ± S.E.	n
Controls	9.72 ± 0.11	18	Controls	10.00 ± 0.10	14
Open Petri dish	10.50 ± 0.27	16	5 µg IAA	9.93 ± 0.07	15
Closed Petri dish	10.83 ± 0.34	18	50 µg IAA	9.79 ± 0.11	14
1 mg IAA	11.40 ± 0.40	10	500 µg IAA	10.42 ± 0.25	14

Table 7.2 The mean node of first initiated flower ±S.E. for lines 58 *(lf e sn hr)*, 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)* grown under continuous light from the start of germination. The seeds were germinated in Petri dishes on cotton wool soaked in water (C) or aqueous solutions of 2, 20 or 100 ppm of Ethrel.

Treatment	L58 $\bar{x}$ ± S.E.	n	L53 $\bar{x}$ ± S.E.	n	L63 $\bar{x}$ ± S.E.	n
C	11.53 ± .41	15	12.94 ± .38	18	12.75 ± .37	8
2 ppm	12.08 ± .34	12	14.12 ± .33	17	- -	0
20 ppm	14.33 ± .36	15	14.61 ± .18	18	14.50 ± .57	8
100 ppm	14.92 ± .15	12	15.06 ± .17	18	15.50 ± .50	2

Table 7.3 The mean node of first initiated flower (FI) ±S.E., length between nodes 1 and 6 (L1-6) ± S.E. and the number of leaves expanded after approximately 25 days growth (LE) ± S.E. for lines 58 *(1f e sn hr)*, 59 *(1f E sn hr)*, 64 *(1f e sn Hr)*, 60 *(1f E Sn hr)*, 53 *(1f e Sn hr)* and 51y *(1f sn Hr)*. The plants were treated either once with 0, 24, 96 or 480 µg of Ethrel or watered with a 100p.p.m. solution of Ethrel every week (E). The photoperiod was 8h.

		58		59		64		60		53		51y	
Character	Treatment	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
FI	0	10.06 ± .06	17	9.06 ± .06	17	9.80 ± .11	15	11.00 ± .00	17	20.53 ± .75	17	9.22 ± .10	18
FI	24/ug	12.44 ± .22	18	10.47 ± .12	17	10.82 ± .36	17	12.06 ± .06	17	21.38 ± .50	13	10.59 ± .15	17
FI	96/ug	14.44 ± .22	18	10.94 ± .10	18	12.13 ± .56	15	12.89 ± .21	18	22.94 ± .44	16	11.06 ± .19	18
FI	480/ug	15.25 ± .18	12	11.69 ± .21	13	13.09 ± .51	11	13.38 ± .14	13	23.13 ± .65	15	12.29 ± .19	17
FI	E	15.17 ± .11	12			13.09 ± .67	11						
L1-6	0	7.56 ± .19	17	8.89 ± .26	14	6.38 ± .10	14	5.11 ± .06	16	5.03 ± .15	17	7.28 ± .12	18
L1-6	24/ug	5.79 ± .11	18	7.45 ± .17	16	5.58 ± .17	17	4.76 ± .17	17	5.37 ± .22	14	5.69 ± .13	17
L1-6	96/ug	5.73 ± .16	18	7.64 ± .12	17	5.57 ± .19	15	4.80 ± .15	17	5.56 ± .22	16	5.53 ± .13	18
L1-6	480/ug	4.82 ± .21	12	6.35 ± .17	13	5.42 ± .22	11	4.61 ± .28	13	4.93 ± .18	11	4.85 ± .15	17
L1-6	E	4.83 ± .17	12			5.38 ± .08	10						
LE	0	6.94 ± .06	17	6.19 ± .10	16	7.13 ± .09	15	7.18 ± .10	17	7.24 ± .11	17	7.00 ± .11	18
LE	24/ug	7.78 ± .10	18	6.76 ± .11	17	7.19 ± .16	16	7.82 ± .10	17	7.79 ± .19	14	6.88 ± .12	16
LE	96/ug	7.39 ± .14	14	6.56 ± .13	16	6.67 ± .16	15	7.44 ± .13	16	7.44 ± .13	16	6.83 ± .09	18
LE	480/ug	7.23 ± .17	13	6.69 ± .13	13	6.18 ± .18	11	6.92 ± .08	13	6.73 ± .23	15	6.29 ± .11	17
LE	E	7.08 ± .23	12			6.73 ± .20	11						

Table 7.4 The mean node of first initiated flower (FI)±S.E. and the length between nodes 1 and 6 (L1-6)±S.E. for lines 58 *(lf e sn hr)*, 59 *(lf E sn hr)* and 68 *(lf e sn hr)* treated with either 0 or 480 µg Ethrel. The percent decrease in the internode length caused by Ethrel treatment is also indicated. The plants received continuous light from the time the plumules broke the surface of the growing medium.

Character	Treatment	L58 $\bar{x}$ ± S.E.	n	L59 $\bar{x}$ ± S.E.	n	L68 $\bar{x}$ ± S.E.	n
FI	0	10.29 ± .11	17	9.11 ± .08	18	9.89 ± .08	18
FI	480 µg	13.25 ± .37	8	11.58 ± .15	12	11.56 ± .18	18
L1-6	0	9.82 ± .25	18	11.77 ± .30	17	7.99 ± .23	17
L1-6	480 µg	4.05 ± .16	8	5.06 ± .27	10	4.58 ± .10	18
Percent decrease L1-6		59		52		43	

Table 7.5 The mean node of first initiated flower ± S.E. for L7 plants (*If*$^{\circ}$ *E Sn hr*) either left intact (C) decotyledonised on day 0 (Decot 0) or day 5 (Decot 5) or treated on the cotyledons with 480 µg of Ethrel prior to germination (Ethrel). The photoperiod was 8h.

C		Decot 0		Decot 5		Ethrel	
$\bar{x} \pm$ S.E.	n	$\bar{x} \pm$ S.E.	n	$\bar{x} \pm$ S.E.	n	$\bar{x} \pm$ S.E.	n
$6.33 \pm .13$	15	$7.38 \pm .11$	21	$6.43 \pm .14$	14	$7.36 \pm .17$	14

Table 7.6 The percentage of L63 (*Lf e Sn Hr*) plants induced to flower by 1 long day (LD) cycle (32h of light) after treatment with either 10 µl of ethanol (C) or 10 µl of ethanol containing 480 µg of Ethrel on either the first or second days before the LD cycle, the day of the LD cycle or the first, second or third days after the LD cycles (treatments -1, -2, 0, 1, 2 and 3 respectively). A further group received 480 ug of Ethrel on each of these 6 days (E). The photoperiod was 8h.

	C	-2	-1	0	1	2	3	E
Percent flowering	89	88	100	93	88	92	100	86
n	19	17	17	14	16	13	12	7

Table 7.7 The table contains the height of the ethylene peak recorded for 1ml samples of air examined using gas chromatography. The air samples came from sealed flasks containing no plants (air), L58 *(If e sn hr)*, L53 *(If e sn hr)* or solutions of 2 or 10 ppm of Ethrel. Both an 8h photoperiod (SD) and continuous light (LD) were used. Three separate runs were carried out, each figure in the table being the mean reading of two air samples for one flask.

Treatment	Run 1	Run 2	Run 3
Air	-	-	.7, .9
L53,SD	4.3, 4.4	1, 1.4	5.4
L58,SD	4.7	2.1, 1.8	5.3
L53,LD	4.0	-	-
Ethrel (2 ppm)	-	-	26.5
Ethrel (10 ppm)	-	-	52.0

Fig. 7.1 Effect of Ethrel on the mean node of first initiated flower of L58 *(lf e sn hr)* plants germinated in open Petri dishes on cotton wool. Vertical bars indicate twice the standard errors; $n = 18$. The plants were exposed to continuous light.

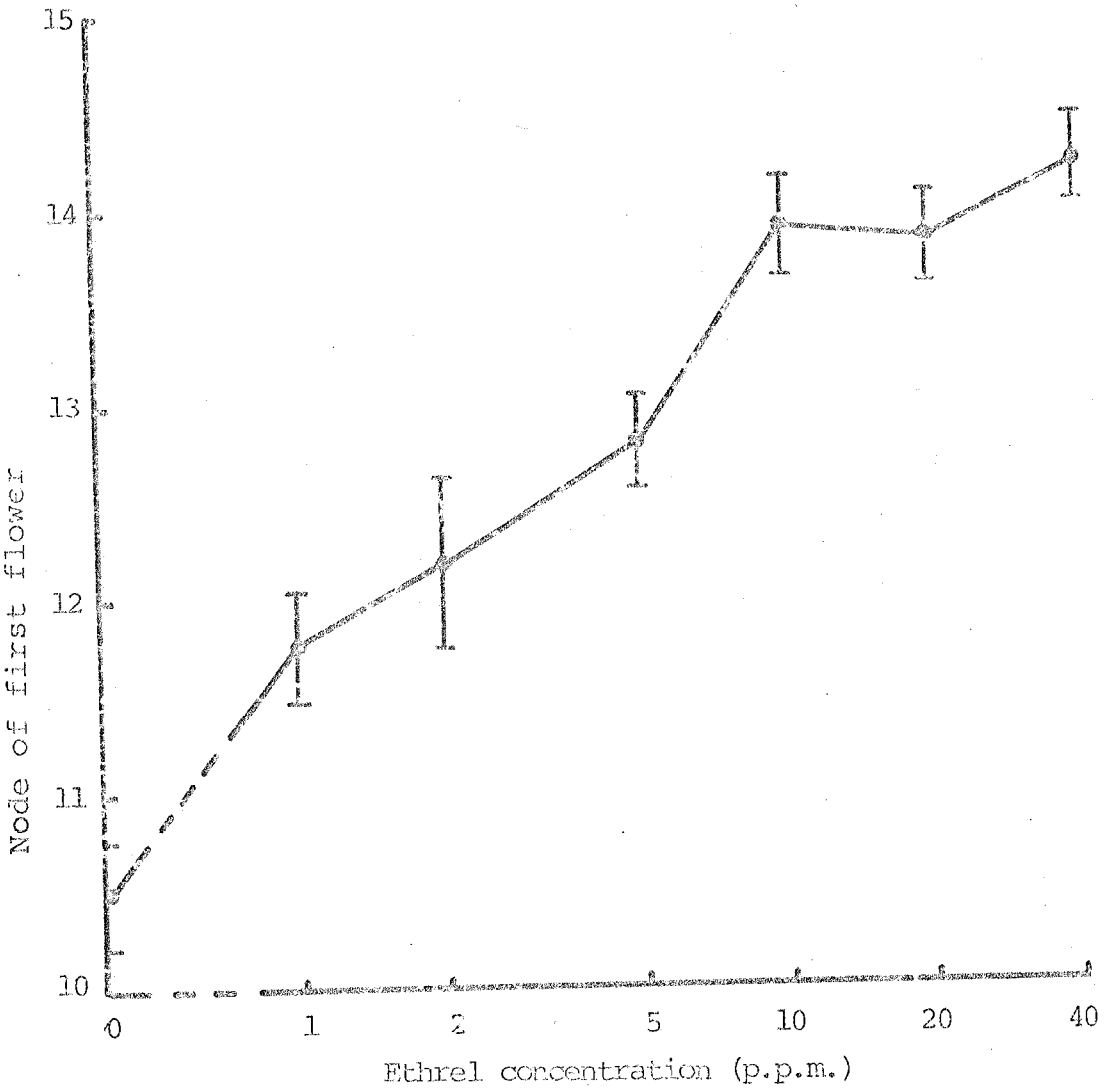
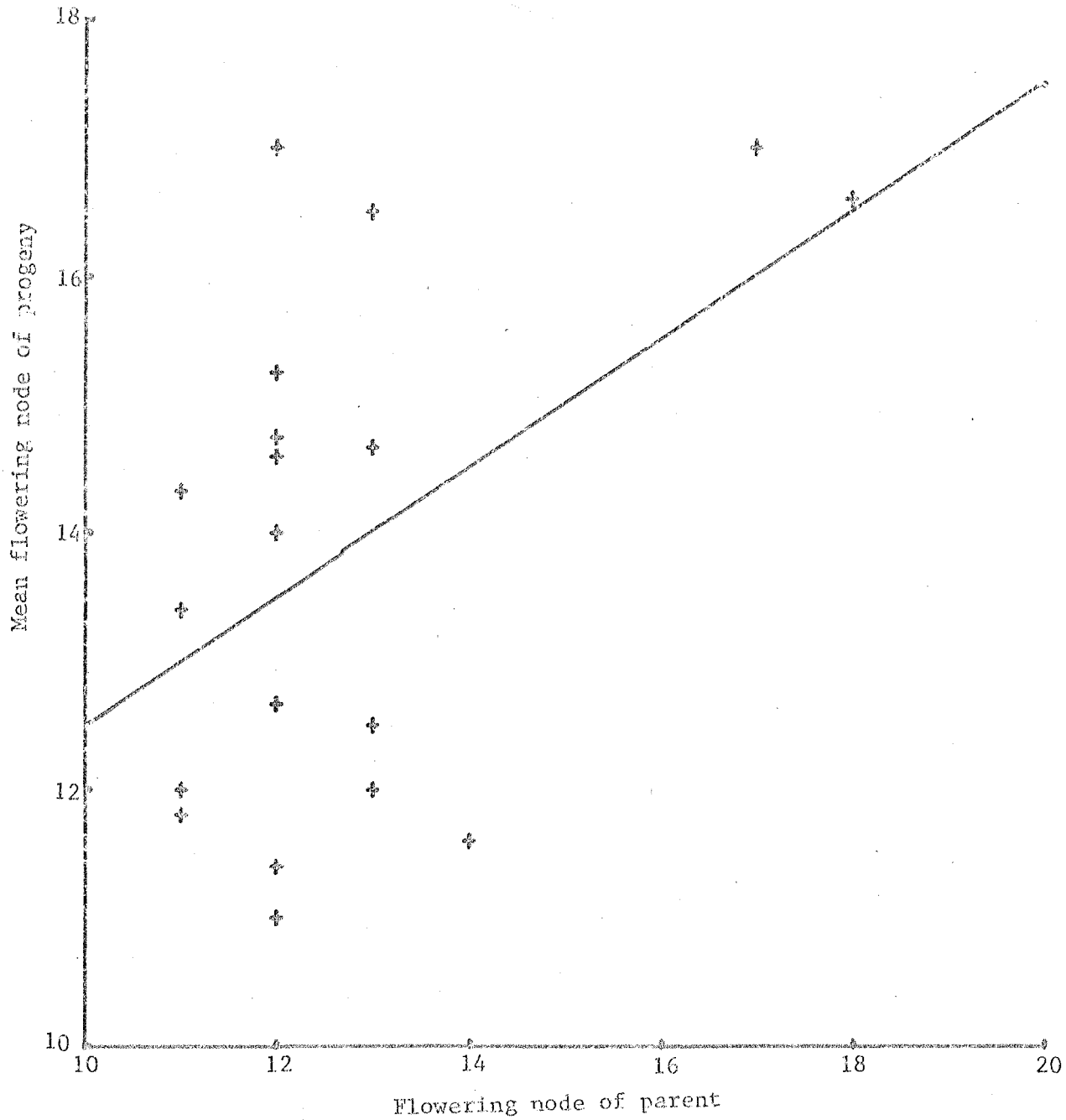

15
14
13
12
11
10
Node of first flower
0
1
2
5
10
20
40
Ethrel concentration (p.p.m.)

Fig. 7.2 Regression of the mean flowering node of progeny of L64 plants *(If e sn Hr)* plotted against the flowering node of the parent ($y = 0.50x + 7.53$). Both the parents and progeny were exposed to an 8h photoperiod and were treated with 480 µg of Ethrel on the cotyledons prior to germination. The slope of the regression is significantly different from 0 (at the 0.05 level).


18
16
14
12
10
Mean flowering node of progeny
10
12
14
16
18
20
Flowering node of parent

CHAPTER 8

GIBBERELLIC ACID

INTRODUCTION

Many attempts have been made to try and determine the physiological role of gibberellic acid (GA_3) in the flowering process of peas. It has been reported to produce anything from a 5 node delay (Dalton and Murfet, 1975) to a 9 node promotion of the flowering node (Wellensiek, 1973b), the size and direction of the response varying with both the genotype and the environment. Dalton and Murfet have shown that plants of the EI phenotype appear to be promoted slightly after treatment with GA_3 while ED and L phenotypes usually show a delay after such treatment although in long photoperiods some L type lines are promoted slightly. The effect in L types was shown to be quite different under LD and SD by these workers, a delay of over 2.9 nodes being observed in all three L lines tested under SD while under LD anything from 1.1 node delay to a 0.6 node promotion was observed. On the other hand, Barber et al. (1958) found the effect of GA_3 on the late cultivars Telephone and Greenfeast to be independent of photoperiod. This difference could have been due to the different dose rates used but would not appear to be due to the genotypes used since Greenfeast seems likely to possess the flowering genotype *Lf e Sn hr* (Murfet, 1976), one of the genotypes used by Dalton and Murfet.

Treatment with GA_3 also affects the flowering time (Barber et al., 1958; Dalton and Murfet, 1975). This results from the change in the flowering node, the abortion of the first flower buds (Brian et al., 1958; Barber et al., 1958; Sprent, 1966b; Wellensiek, 1973b) and an increase in the rate of node expansion

(Brian et al., 1958; Sprent, 1966b; chapter 6). These effects may either cancel each other out or be additive depending on the effect of GA_3 on the flowering node. For example, Dalton and Murfet found in LD that 5 µg of GA_3 delayed the flowering node by 1 node but decreased the flowering time by half a day in L24. This has led to some controversy over whether GA_3 has a direct effect on flowering (Collins and Wilson, 1973a) even though Barber et al. (1958) indicated that GA_3 delayed the flowering node, time to open flower and time of initiation in the ED type Massey. When L61a plants were treated with GA_3 under SD conditions the penetrance was not significantly altered although the flowering node of the plants in the late region was significantly delayed (chapter 6). This result would suggest that GA_3 is not directly altering the ratio of promotor to inhibitor or the ratio of promotor to inhibitor required for flowering. However it is possible that GA_3 may act by altering the aging processes which results in the alteration of the ratio of promotor to inhibitor at a fairly late stage in the life of the plants.

The present work set out to examine the interaction between the photoperiod length and GA_3 in the genotypes *Lf e Sn hr*, *lf e Sn Hr* and *lf e Sn hr*. It was hoped to ascertain whether effects were direct ones on the flowering process by the measurement of three different parameters of flowering, the flowering node, flowering time and time of flower initiation. The age at which application was effective was also examined in order to show whether GA_3 could possibly be acting by altering the aging processes. Further, the effect of GA_3 on the genotypes *lf e sn hr* and *lf e sn Hr* was examined since the gene *Hr* has been shown to affect the aging

process (Chapter 5) and consequently the relationship between *Hr* and GA_3 is of considerable interest.

MATERIALS AND METHODS

Determination of the Time of Initiation in L24 (*Lf e Sn hr*).

The experiment consisted of 2 photoperiods, 8h light (SD) and continuous light (LD), and 3 chemical treatments, either 10 µl of ethanol placed on the testa before planting and then 10 µl of ethanol placed on the first expanded leaf back from the apex every 2 weeks from day 13 until day 55 (controls), 10 µl of ethanol containing 10 µg of GA_3 placed on the cotyledons and then 10 µl of ethanol each fortnight as in the previous treatment (GA_3) or 10 µl of ethanol containing 10 µg of GA_3 on the cotyledons and on the first expanded leaf back from the apex each fortnight from day 13 until day 55 (GA_3 cont.). Seventy two plants of each treatment were planted under SD and 54 under LD. Each week, until day 34 for LD and day 62 for SD plants, 6 plants of each treatment (sometimes less due to the death of some plants) were dissected and the total number of nodes, the flowering node (if present) and the number of expanded leaves were recorded. A similar group of plants were dissected on day 20 for the LD treatments and on day 1 for the controls. A group of 18 plants of each treatment were allowed to mature from which data were obtained on internode length, the flowering node, the flowering time and the number of leaves expanded at various times. The results from this experiment are contained in table 8.1 and figs. 8.1 and 8.2.

Determination of Age at which Treatment with GA_3 is Effective in L24.

All plants were grown in an 8h photoperiod and either treated with 10 µl of ethanol on the testa before planting and

on the first fully expanded leaf back from the apex of days 29 and 39 (controls), treated in a similar manner as the controls except the ethanol contained 10 µg of GA_3 on either day 0 (GA_3D0), day 29 (GA_3D29) or day 39 (GA_3D39), or treated with ethanol containing 10 µg of GA_3 on the testa and every second week until day 70 (GA_3 cont.). The number of leaves expanded were recorded on days 29, 39 and 55, the results for the experiment being tabulated in table 8.2.

Determination of Response of Lines 53 and 63 to GA_3.

The experiment was of a factorial nature and involved two lines, L53 *(lf e Sn hr)* and L63 *(lf e Sn Hr)*, two photoperiods, 8h light and 24h light, and two chemical treatments, either 10 µl of ethanol applied to the dry testa before planting and to the last fully expanded leaf on days 13 and 27 (controls) or a similar treatment with the ethanol containing 10 µg of GA_3 (GA_3). Impenetrant plants were excluded from the analysis under SD. The results are contained in table 8.3.

Response of Genotypes *lf e sn hr* and *lf e sn Hr* to GA_3 and Gibberellin Synthesis Inhibitors.

The two lines, L68 *(lf e sn hr)* and L64 *(lf e sn Hr)* were exposed to either an 8h photoperiod or continuous light and either left intact or had their cotyledons removed on days 6 and 7. The plants exposed to an 8h photoperiod were treated either with 10 µl of ethanol on the testa before planting or in a similar way with the ethanol containing either 10 µg of GA_3, 100 µg of AMO 1618, or 1000 µg of CCC. AMO 1618 and CCC have both been reported to inhibit the synthesis of gibberellins in plants (Cathey, 1964). In continuous light only control plants and GA_3 treated plants were grown. The results are tabulated in table 8.4 and Appendix 1.

RESULTS

L24 plants exposed to an 8h photoperiod show a substantial delay in the flowering node when treated with 10 μg of GA_3 on day 0. This delay was 5.11 nodes in one experiment (table 8.1) and 3.87 nodes in another (table 8.2)(both delays were significant at the 0.001 level). Similar treatment under continuous light led to a small 0.53 node delay (significant at the 0.05 level), the interaction between GA_3 and photoperiod being similar to that reported by Dalton and Murfet (1975). Continuous treatment with GA_3 increased the delay under an 8 h photoperiod (further delays of 2.31 and 3.53 nodes being recorded) but in continuous light resulted in an insignificant promotion of the flowering node compared to the plants given a single dose of GA_3. The effect of these treatments on the flowering time varied from a promotion of 2.3 days with a single application under continuous light (significant at the 0.001 level) to a delay of 2.2 days by a similar treatment under an 8h photoperiod (significant at the 0.05 level). Continuous application did not significantly alter the flowering time under either photoperiod since the increased rate of leaf expansion cancelled out the delays in the flowering node.

The time at which the first flower bud was initiated was almost identical in all three treatments in continuous light (fig. 8.1) but differed substantially under SD conditions where the plants treated with a single dose of GA_3 initiated 4.5 days after the untreated plants while those given several doses of GA_3 initiated 6 days after the untreated plants (fig.8.2). This delay in the time of initiation was even more apparent in the plants dissected where 3 out of 5 untreated plants had observable flower buds on day 55 (and all did by day 62), while none of the

5 continuously treated plants had observable flower buds on day 55 or 62. The number of leaves expanded at the time of flower initiation differed under both photoperiods, the untreated plants having approximately 1 less leaf expanded in continuous light and 5 to 7 less in SD than the plants treated with GA_3. These results would suggest that GA_3 can directly affect the flowering process in the late line, L24 *(Lf e Sn hr)*, under the 8h photoperiod since both the flowering node and the time of initiation are substantially delayed. The time of open flower is also significantly delayed by a single application but this character would seem a poor indicator of the changeover from the vegetative to the reproductive state since it can be affected markedly by flower abortions and the growth rate (including the post-initiation growth rate). Since the number of leaves expanded at the time of initiation was substantially altered the leaf requirements for flowering must be altered by the treatment with GA_3 and this rules out the possibility that GA_3 operates by increasing the number of unexpanded leaves in the apex when the leaf requirement for flowering is met. If anything treatment with GA_3 reduces the number of unexpanded leaves in the apex at least during the early stages of growth. Under continuous light, GA_3 appears to have little direct effect on the flowering process in L24, although some small, but significant, alterations in the flowering node and time were observed.

L24 plants exposed to SD conditions vary substantially in their response to a single 10 µg dose of GA_3 as they become older (table 8.2). When the GA_3 was applied at day 0 it resulted in a 3.83 node delay in the flowering node (significant at the 0.001 level) while at day 29 it caused only a 1.56 node

delay (significant at the 0.05 level) and at day 39 only a 0.6 node delay (insignificant). The reduced effect of GA_3 with age cannot be due to initiation already having occurred as dissections on day 39 showed that only 23.5±.5 nodes were present. The later applications did reach a site where they could exert an effect since they caused a 400% increase in internode length (table 8.2). It would therefore appear that GA_3 can only be fully effective if it is present well before the time of flower initiation.

In both L53 and L63 GA_3 caused small (up to 1 node) but significant (at the 0.01 level) promotions of the flowering node in continuous light (table 8.3). The time of flower initiation would also have been promoted (by between 2 and 4 days) if GA_3 is assumed to alter the vegetative growth of L63 and L53 plants in a similar way to the L24 plants shown in fig. 8.1 . Under SD conditions GA_3 significantly delayed the flowering node of L53 (at the 0.001 level) but was ineffective in L63 since the plants do not flower until after transfer to long photoperiods. GA_3 delayed the change from two to more than two leaflets per leaf in both L63 and L53 under both photoperiods. This result was somewhat unexpected since there is usually a strong positive correlation between the flowering node and the change to more than two leaflets per leaf (Barber, 1959).

GA_3 consistently delayed the flowering nodes of lines 68 and 64 regardless of the length of the photoperiod or whether the cotyledons were removed or not. The delays varied from 0.1 to 4.5 nodes and were significant in all cases except for L68 plants under an 8h photoperiod (table 8.4). Treatment of the same two lines with the supposed inhibitors of gibberellin synthesis,

AMO 1618 and CCC, did not significantly alter the flowering node of either intact or decotyledonised plants under SD (table 8.4). The size of the delays caused by GA_3 varied considerably between lines, L64 being delayed to a larger extent than L68, especially under SD conditions. This difference probably reflects the fact that the hormonal balance in L64 is close to the threshold for flowering for quite a long period whereas L68 plants pass the threshold for flowering rapidly and steeply. Consequently a small shift in either the threshold or the hormonal balance will cause a much larger response in L64 than in L68. The closeness of L64 to the threshold for flowering was clearly shown in the decotyledonised plants under SD where bimodal distributions of the flowering node were observed (see Appendix 1 for the distribution of the flowering nodes). Only 1 plant out of 68 plants flowered at node 11 the rest flowering either at nodes 9 and 10 or at 12 to 19. No such bimodality was observed in similarly treated L68 plants. The only treatment which affected the distribution of L64 plants into these two groups was GA_3 which caused all plants to flower in the later group further illustrating the delaying action of GA_3. The mean flowering nodes for the L64 plants in the later group showed considerable variation but again only GA_3 caused a significant delay (at the 0.05 level) when compared to the controls. Consequently the statistical results obtained from the total data are in agreement with those from only the later group and therefore no special statistical tests were used to overcome the bimodality of the data.

DISCUSSION

It is well established that GA_3 can produce a large and significant delay in the flowering node of late cultivars

under SD conditions. The present results indicate this effect does not occur primarily through an alteration of the growth rate or the number of unexpanded leaves in the apex and suggest that, for a maximal effect, treatment with GA_3 needs to occur at an early stage in the growth of the plant. At the time initiation would occur any positive effect of GA_3 on internode length had disappeared in the plants treated on day 0 and therefore the results would suggest that GA_3 must permanently alter either the threshold ratio of the flowering hormones required at the apex for flowering or the level of promotor or inhibitor. Since GA_3 is able to promote the flowering node (and presumably the time of initiation) of both EI and L varieties under LD the first alternative would appear unlikely. Since the penetrance of L61a is not significantly altered by GA_3 and GA_3 treatments are not effective just prior to initiation in L24 it would appear GA_3 does not have an immediate effect on the level of promotor or inhibitor. It is suggested GA_3 reduces the rate of the aging response of *Sn* and in order to exert this effect it needs to be present during the development of the plant (leaves). Whether the reduction of the leaf area by GA_3 (Dalton, unpub.) plays a part in the response is not known but from the results of leaf removal experiments it would not appear that this could be of prime importance.

GA_3 can mimic the effects of the length loci *La* and *Cry* on internode length and the flowering node (Murfet, 1971b; Dalton and Murfet, 1975). This would suggest a relationship between the length genes and gibberellin metabolism. All that needs to be added is that the reason for the later flowering of late flowering cryptodwarf genotypes (compared to late

flowering dwarf genotypes) is possibly due to a reduction in the rate of the aging processes in the shoot which could result from increased levels of gibberellins. However, AMO1618 does not cause cryptodwarf plants to become phenocopies of dwarf plants (Reid, unpub.) suggesting that increased production of gibberellins may not be responsible for the increased internode length in these plants. These results are not easily reconcileable but are similar to the results of McComb and McComb (1970). Brian (1957) and Murfet (1976) both discuss theories which account for these confusing results.

It does not appear that the gene *Hr* operates via the gibberellins even though this gene has been shown to reduce the effect of age on the gene *Sn* since applied GA_3 and the supposed inhibitors of gibberellin synthesis, AMO 1618 and CCC did not appear to reduce the phenotypic difference between L68 *(lf e sn hr)* and L64 *(lf e sn Hr)*. This difference is poorly illustrated by the flowering node data in table 8.4 but could be observed by comparing the flowering time and total number of leaves expanded. Repeated treatment of L63 plants with AMO 1618 under SD conditions also did not cause initiation as might be suspected if the gibberellins are responsible for the reduced effect of age on the gene *Sn* by the gene *Hr* (Reid, unpub.). Also a progeny segregating for the *Hr* gene showed no pleiotropic action of the *Hr* gene on internode length (Murfet, unpub.). However, these results do not completely preclude the gibberellins (other than GA_3) from being involved with the gene *Hr* since several gibberellins (e.g. GA_8, GA_4, allogibberic acid) do not cause large alterations in the internode length of peas while still affecting flowering (Murfet and Barber, 1961; Dalton, unpub.) and it appears doubtful that either AMO 1618

or CCC completely inhibits their synthesis. Direct measurements of the gibberellins may be the only method of completely answering the question.

When treated with GA_3 the flowering responses of ED and EI lines under both LD and SD and of LHR and L lines under LD are small and varied. ED lines show a small delay in the flowering node while EI lines show a small promotion (Barber et al., 1958; Dalton and Murfet, 1975). Of the L and LHR lines for which data are available, Greenfeast, Telephone and L24 *(Lf e Sn hr)* show a small delay while lines 2 *(Lf E Sn hr)*, 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)* show a small promotion in the flowering node (Barber et al., 1958; Dalton and Murfet, 1975; table 8.3). In L24, however, the time to initiation is not altered by the treatment. The only consistent factor in these results is that promotion only occurs if *Sn* is present. In lines carrying *Sn* the young shoot produces a more inhibitory ratio of the flowering hormones than the cotyledons (chapter 6) this being especially evident in lines which also carry the gene *E* (Murfet, 1973b). It is suggested that treatment with GA_3 may increase the cotyledonary influence relative to that of the shoot over the early stages of growth and this leads to a promotion of the flowering node and also possibly in the time of initiation in EI and some of the L and LHR lines. However, the reason for the small delays in the flowering nodes of other L lines and all the tested ED lines remains unclear. It appears further work on the interaction of GA_3 with the genes controlling flowering is called for before firm proposals as to the mode of action of GA_3 can be made.

Table 8.1 The mean node of first initiated flower (FI) ± S.E., number of days to the first open flower (FT) ± S.E., node of first open flower (FD) ± S.E. and total number of leaves expanded by the plant (TNE) ± S.E., for L24 plants *(Lf e Sn hr)* exposed to either continuous light or an 8h photoperiod. The plants received no GA_3 (Control), a single 10 µg dose of GA_3 prior to imbibition (Day 0) or a 10 µg dose of GA_3 every two weeks (Continuous). The smallest number of plants scored was sixteen. Significance levels indicate differences from the respective control treatments.

CHARACTER	Continuous Light			8h Light		
	CONTROL $\bar{x}\pm$S.E.	DAY 0 $\bar{x}\pm$S.E.	CONTINUOUS $\bar{x}\pm$S.E.	CONTROL $\bar{x}\pm$S.E.	DAY 0 $\bar{x}\pm$S.E.	CONTINUOUS $\bar{x}\pm$S.E.
FI	17.18±.16	17.71±.11^{x}	17.56±.16	24.64±.33	29.75±.57^{xxx}	32.06±.45^{xxx}
FT	60.65±.31	58.35±.41^{xxx}	60.61±.78	88.24±.87	90.44±.61^{x}	89.44±.61
FD	17.18±.16	17.71±.11^{x}	18.72±.27^{xxx}	24.64±.33	29.75±.57^{xxx}	32.06±.45^{xxx}
TNE	18.47±.23	19.65±.23^{xx}	21.89±.31^{xxx}	25.59±.32	30.81±.65^{xxx}	32.90±.47^{xxx}

Table 8.2 The mean node of first initiated flower (FI) ±S.E.,number of leaves expanded (LE) on days 29, 39 and 55 ±S.E., and length between nodes 1 and 6(L1-6), 6 and 10(L6-10), 10 and 14(10-14) and 14 and 20(L14-20) for L24 plants *(Lf e Sn hr)* exposed to an 8h photoperiod. The plants either received no GA_3 (Control), 10µg of GA_3 on either day 0, 29 or 39 or 10µg of GA_3 every 2 weeks (Continuous). The smallest number of plants scored was 20. Significance levels indicate differences from the relevant control treatment.

CHARACTER	FI	LE on day 29	LE on day 39	LE on day 55	L1-6	L6-10	L10-14	L14-20
TREATMENT	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.
CONTROL	26.40 ± .48	9.10 ± .07	12.95 ± .09	19.10 ± .14	5.81 ± .11	8.10 ± .21	8.30 ± .17	16.49 ± .43
DAY 0	30.17 ± .42***	10.30 ± .17***	14.14 ± .18***	19.91 ± .26**	30.33 ± 1.20***	11.68 ± .83***	7.70 ± .23*	13.17 ± .43***
DAY 29	27.96 ± .49*	8.68 ± .12**	13.23 ± .20	20.76 ± .28***	5.92 ± .20	13.53 ± .89***	37.20 ± .75***	50.49 ± 2.50***
DAY 39	27.00 ± .35	9.10 ± .07	12.81 ± .13	19.95 ± .22**	5.77 ± .10	8.07 ± .31	12.60 ± .68***	63.27 ± 1.05***
CONTINUOUS	33.70 ± .32***	11.00 ± .09***	15.88 ± .17***	22.95 ± .20***	29.34 ± 1.20***	29.44 ± .96***	20.86 ± .66***	44.11 ± 1.55***

Table 8.3 The mean node of first initiated flower (FI) ± S.E., number of days to first open flower (FT) ± S.E., node of first pod (FP) ±S.E. and first node with more than 2 leaflets (L>2) ± S.E. for lines 63 *(lf e Sn Hr)* and 53 *(lf e Sn hr)*. The plants were either left untreated (Control) or treated with GA_3 and either exposed to an 8h photoperiod (SD) or to continuous light (LD). The smallest number of plants scored was 16. Significance levels indicate differences from the relevant control treatment.

	L53				L63			
	LD		SD		LD		SD	
Character	Control	GA_3	Control	GA_3	Control	GA_3	Control	GA_3
	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.
FI	14.22 ± .14	13.38 ± .23**	21.28 ± .37	24.24 ± .53***	14.65 ± .19	13.63 ± .22**	44.69 ± .69	44.87 ± .95
FT	34.95 ± .44	32.24 ± .30***	61.88 ± 1.19	60.00 ± .98	37.26 ± .42	36.87 ± .62	-	-
FP	14.26 ± .14	13.95 ± .22	22.21 ± .37	24.84 ± .65**	14.65 ± .19	14.44 ± .33	-	-
L>2	12.00 ± .27	13.95 ± .35***	16.17 ± .60	22.26 ± .51***	15.09 ± .32	16.63 ± .24**	18.56 ± .72	24.06 ± .73***

Table 8.4 The mean node of first initiated flower ± S.E. for lines 64 *(lf e sn Hr)* and 68 *(lf e sn hr)* exposed to either continuous light (LD) or an 8h photoperiod (SD) and either left intact or decotyledonised on days 6 and 7 (-). Plants were treated with either 10 µg of GA_3, 100 µg of AMO 1618 or 1000 µg of CCC prior to imbibition or left untreated (Control). Significance levels indicate differences between the various chemical treatments and the relevant control treatment.

Treatment	Control		GA		AMO1618		CCC	
	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
L64,SD, INTACT	9.95 ± .12	19	12.40 ± .81 ***	5	9.94 ± .25	16	10.06 ± .10	18
L64,SD, -	11.84 ± .65	19	16.33 ± .41 ***	12	12.06 ± .47	18	13.16 ± .79	19
L64,LD, INTACT	9.95 ± .19	19	11.07 ± .22 ***	14	-		-	
L64,LD,-	9.94 ± .06	18	11.42 ± .19 ***	12	-		-	
L68,SD, INTACT	10.41 ± .15	17	10.90 ± .31	10	10.24 ± .18	17	10.13 ± .18	16
L68,SD,-	11.38 ± .27	16	11.50 ± .22	10	10.95 ± .24	19	10.79 ± .14	21
L68,LD, INTACT	10.26 ± .15	15	11.18 ± .38 *	11	-		-	
L68,LD, -	10.20 ± .12	20	11.29 ± .24 ***	14	-		-	

Fig. 8.1 Graphs of total nodes (————) and number of leaves expanded (- - -) versus age for L24 plants *(Lf e Sn hr)* exposed to continuous light and given either no GA_3 (+), 10 µg of GA_3 prior to imbibition (0) or 10 µg of GA_3 every two weeks (□). The flowering node (x) and time of flower initiation (. . . .) is indicated for each treatment.

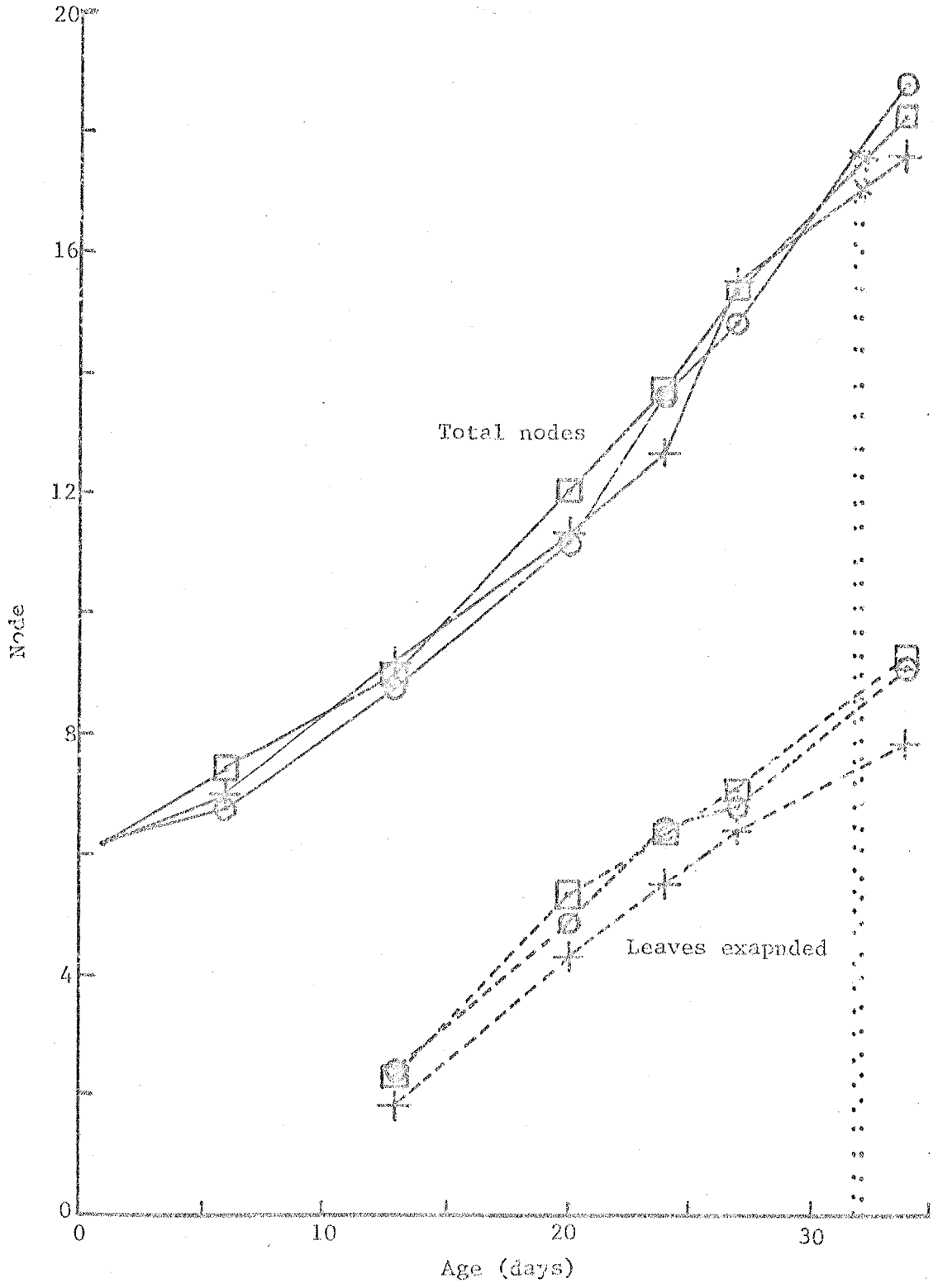
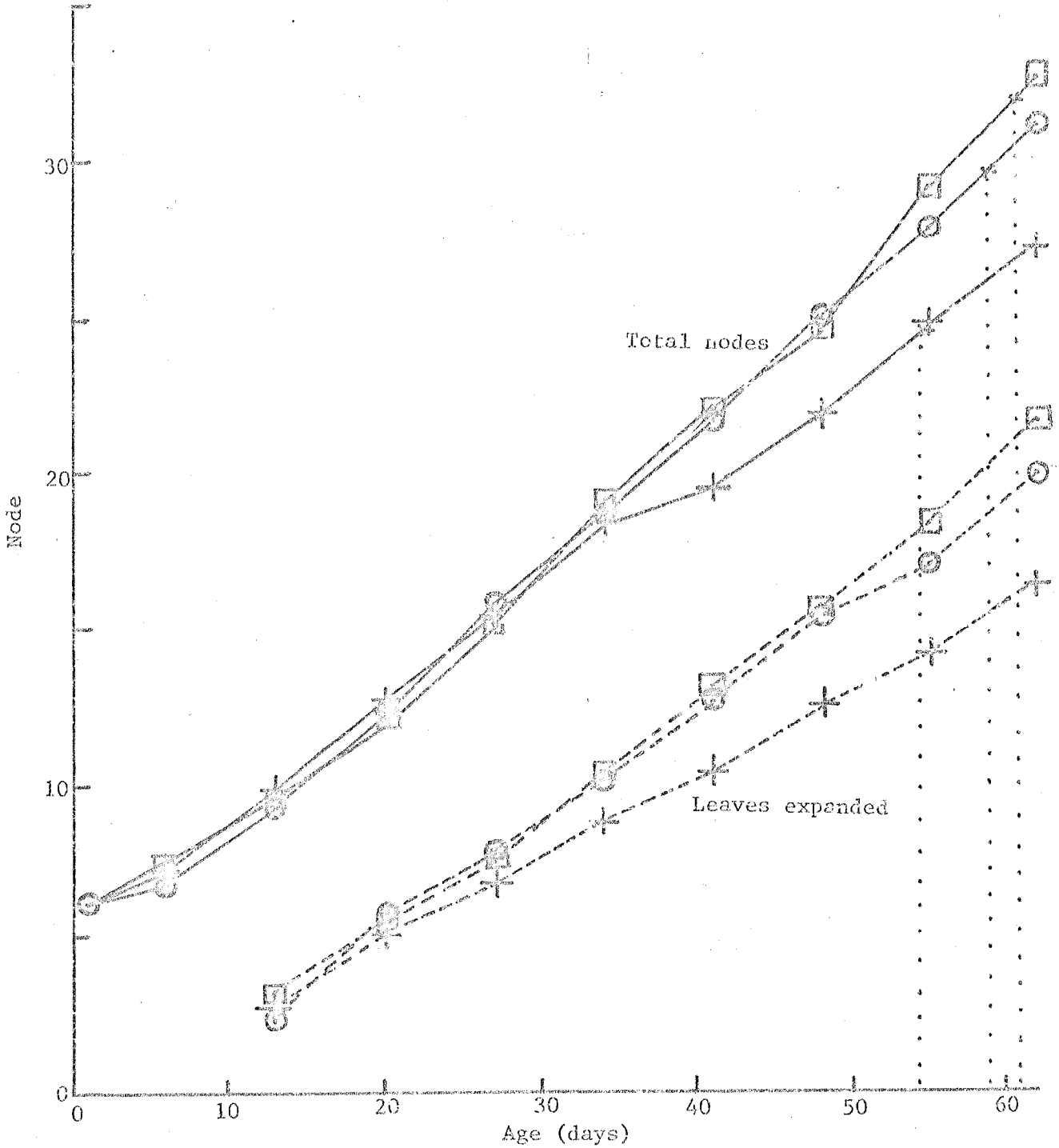

20
16
12
8
4
0
Node
Total nodes
Leaves exapnded
0
10
20
30
Age (days)

Fig. 8.2 Graphs of total nodes (————) and number of leaves expanded (- - -) versus age for L24 plants *(Lf e Sn hr)* exposed to an 8h photoperiod and given either no GA_3 (+), 10 µg of GA_3 prior to germination (0) or 10 µg of GA_3 every two weeks (□). The flowering node (x) and time of flower initiation (. . . .) is indicated for each treatment.


Node
30
20
10
0
0
10
20
30
40
50
60
Age (days)
Total nodes
Leaves expanded

CHAPTER 9

GENERAL DISCUSSION AND PROPOSED MODEL

DISCUSSION

All that remains to be done is to develop an integrated model of the control of flowering in peas, especially in the genotypes *Lf e Sn hr*, *lf e Sn Hr*, *lf e sn Hr* and *lf e sn hr*. This is done on the following pages and is diagramatically illustrated in figs. 9.1 and 9.2. It is hoped the figures will enable the prediction of the flowering behaviour of various genotypes of peas under a number of different environmental conditions. It should be noted that the lines on these figures represent the proposed mean for a group of plants and consequently would be expected to have a variance due to developmental noise in individual plants. This may lead to some plants of a genotype behaving differently to others since some plants may just pass a threshold while the others do not (e.g. fig. 9.2, L61a, SD). The development of the model draws heavily on the published work of other workers, especially Murfet (1971b, c, 1973a,b, 1975), as well as on the drawing together of the relevant results from the experimental chapters of this thesis.

The change from vegetative to reproductive growth in peas appears to be controlled by the ratio of a flower promoting to a flower inhibiting substance (Murfet, 1971c, 1976). However, much controversy over this point has occurred, some workers suggesting that only an inhibitor needs to be postulated (Paton and Barber, 1955; Barber, 1959; Amos and Crowden, 1969; Sprent and Barber, 1957) while others suggest that only a promotor needs to be postulated (Haupt, 1957, 1969; Köhler, 1965). The strongest evidence for the involvement of an inhibitor comes from the delay in the flowering node of 58/53 grafts compared to 58/58 self

grafts or decotyledonised L58 plants (Murfet and Reid, 1973), while that for the existence of a promotor comes from the flowering of late scions in the early region when they are grafted onto early stocks (Köhler, 1955; Murfet, 1971c). Decotyledonised late varieties still flower in the late region although a small promotion of the flowering node is observed which appears to be attributable to the reduced growth rate of this treatment (Haupt, 1969; Murfet, 1973b; Amos, 1974, chapter 5).

The production of inhibitor appears to be controlled by the *Sn* gene (Barber, 1959; Murfet, 1971b) and can occur in both the shoot and cotyledons (Murfet, 1971c, 1973b). The expression of the *Sn* gene can be markedly altered by both the photoperiod and temperature. Under some conditions these factors may even eliminate the difference between genotype *If e sn hr* and *If e Sn hr*, provided appropriate genetic backgrounds are present. The gene *sn* does not appear to be entirely inactive since photoperiod effects can be shown in decotyledonised plants of the genotypes *If e sn hr* and *If e sn Hr*. The largest effects are in the genotype *If e sn Hr* and this probably reflects the modification of the activity of the gene *sn* by the gene *Hr*. The difference between the *Sn* and *sn* alleles may be minor, the activity of the product of *sn* being only partially impaired by the change in the structure of the gene.

The genetic control of the production of promotor is not known, the level appearing to be similar in all the genotypes used, although this does not mean that variations in the level of promotor production do not occur. Some of the polygenic modifier systems described by Murfet (1971a, 1971b, 1973a, 1975) and

Rowberry (unpub.) may well act by altering the level of promotor and in the future major genes affecting the level of production may be found. However, in several distinct areas (e.g. enzyme polymorphisms, plant hormones, plant genetics) workers have suggested that the more "important" a particular enzyme, hormone or gene the less likely it is to be found varying widely between individuals. Consequently if the promotor is an important metabolite in the plant and is therefore involved in many developmental sequences any major change in its level of production may be lethal and consequently a major locus involved with its production may not be found.

Two alternative systems for the control of flowering seem to exist once the existence of both a flower promotor and a flower inhibitor has been established. The hormones may either act independently of each other, each requiring to pass a threshold before flowering can commence, or they may interact, flowering not occurring until the ratio of promotor to inhibitor passes some threshold. This latter system has been termed the balance model by Murfet (1971c) and has been used extensively by him. It has been used throughout the present study since it quite adequately describes the experimental results and has been shown to have wide application in the control of other developmental sequences in plants (Skoog and Miller, 1957; Galston and Davies, 1970). Further the fact that altered vegetative growth (e.g. by cotyledon removal) only marginally alters the flowering process in some lines would support the balance model since quite large alterations in the absolute quantities of the hormones would be expected. However, without a knowledge of the chemicals involved and consequently a means of directly measuring their concentrations it is impossible to exclude the independent

threshold model.

It is the ratio of promotor to inhibitor and/or the threshold of this ratio required for flowering which are altered by treatments which cause what have been termed "direct" effects on the flowering process. It is often extremely difficult to show which of the three variables has been altered and it is probably this point, more than any other, which has been pursued in the present work. For example, light can be readily shown to lower the flowering node of L and LHR types of peas. Barber (1959) suggested light destroyed an inhibitor, while Amos (1974) suggested that light controlled the amount of promotor produced in the leaves, this difference in promotor levels only being observable in the presence of a cotyledonary inhibitor produced by the gene *Sn*. The present results (Chapter 3) would suggest light reduces the production of inhibitor by the *Sn* gene in both the shoot and cotyledons thus raising the promotor to inhibitor ratio. Consequently the threshold for flowering is passed more quickly in continuous light than in an 8h photoperiod (figs. 9.1a and 9.1c) A second example is that vernalisation and continuous light can cause almost identical changes in the flowering node if in both cases the control plants are exposed to warm SD conditions (e.g. L61a, chapter 6). However, the mechanisms by which these changes occur appear to be completely different. The probable mechanism by which light operates is stated above while vernalisation and cool temperatures are thought to raise the ratio of promotor to inhibitor because their formative reactions possess different temperature coefficients. This implies that the absolute quantitities of both substances are altered. As well as altering

these levels it has also been suggested that vernalisation lowers the threshold ratio required for flowering and may also alter the aging processes (chapter 4). How such a model would cause the observed alterations in the flowering nodes of L61a and L63 is summarised in figs. 9.2b and 9.2c.

The aging responses have been shown to occur in the leaves and appear to be caused by the reduction in the activity of the *Sn* gene as the plant ages. The gene *Hr* appears to reduce the size of this response in plants carrying both *Sn* and *sn* (figs 9.1a and 9.1b) but it is not clear if *Hr* also affects the flowering process in any other way. Plants carrying *Hr* appear to show a tendency for greater post-reproductive growth at least in photoperiods of less than 24h. This effect can be attributed to a lower promotor to inhibitor ratio which the author considers plays a significant role in the development of the flower buds once they have initiated and in the senescence of the plant. Some of the quantitative systems which influence flowering also appear to act by reducing the aging response of *Sn*. Included in these systems are the pleiotropic effects of the alleles at the *La* and *cry* loci on flowering (Dalton and Murfet, 1975), their suggested effect on the promotor to inhibitor ratio being illustrated by a comparison of L53 and L61a above node 17 in fig. 9.2a. The proposed effect of the penetrance modifiers in the genotype *lf e Sn hr* (Murfet, 1973b) is also illustrated in this figure along with the temporary decline in the promotor to inhibitor ratio after node 16. This reduced ratio appears to come about due to the lower ratio of promotor to inhibitor reaching the apex from the shoot after the senescence of the cotyledons after approximately 3 weeks growth. Reversion

to the vegetative state might be expected to occur in all the impenetrant L61a plants at about node 16 since the ratio of hormones is indicated to fall below the threshold for flowering (fig. 9.2a) even after allowing for developmental noise in individual plants. Although some plants do show vegetative reversion it is not frequent in impenetrant L61a exposed to an 8h photoperiod suggesting that for reversion to occur possibly either a second lower threshold needs to be passed or that the already initiated flower primordia produce a promotory level of flowering hormones. Support for one of these alternatives comes from the continued flowering (up to 40 nodes) of L63 plants induced by a single LD cycle when clearly the ratio of promotor to inhibitor would be expected to have fallen back to that of the non-flowering plants in SD. However some treatments do cause complete vegetative reversion. For example, growth of vernalised L63 plants at 30^{o}C after initiation in the early region caused the promotor to inhibitor ratio to be reduced to such an extent that vegetative reversion occurred in all plants which had previously flowered (chapter 4).

The actions of the different alleles at the *e* and *lf* loci on the model presented have not been examined extensively during the present work. The work of Murfet (1971c, 1973b) clearly shows that *E* causes an increase in the ratio of promotor to inhibitor leaving the cotyledons in plants carrying *Sn* but whether this is due to a decrease in the level of inhibitor or an increase in the level of promotor is not known. Plants carrying *e* also appear to produce higher promotor to inhibitor ratios in their cotyledons than in the young shoots as is shown by the increased penetrance in L61a *(lf e Sn hr)* and the delayed flowering node in L64 *(lf e sn Hr)* after decotyledonisation

on day 5. The actual level of the ratio produced in e cotyledons can vary significantly depending on the remaining genetic background, as is shown by a comparison of L53 and L61a (both *lf e Sn hr*) (fig. 9.2a).

The *lf* locus has been suggested to influence the threshold ratio of promotor to inhibitor required for flowering (Murfet 1971b, 1971c, 1975). Four alleles have so far been identified at this locus and cause later flowering in the order lf^a, *lf*, *Lf*, Lf^d (Murfet, 1975). At first these alleles appear to define the length of a juvenile phase, plants carrying *Lf* not flowering below node 11 under any environmental or genetic circumstances, those with *lf* not below node 8 and those with lf^a not below node 5. However the *Lf* and *lf* alleles do affect the sensitivity of plants to LD cycles even in the late region (chapter 3) giving support to the view (Murfet, 1971b) that the locus controls a threshold that lasts throughout a plant's life, not just a juvenile phase. It is this view that has been taken in the construction of the models illustrated in fig. 9.1. Although the 4 alleles have been drawn to give 4 thresholds equidistant apart the scale indicated for the promotor to inhibitor ratio is completely arbitary and it is not suggested that the 4 alleles in fact give thresholds which are equidistant apart.

Many of the genotypes possible with the 4 loci have not been observed but some interesting predictions could be made by the use of fig. 9.1. For example under SD it may be possible to obtain a clear segregation of the flowering node between plants possessing *hr* and *Hr* on a *sn* background if either the *Lf* or Lf^d alleles were present (fig. 9.1a).

With a *lf sn* background such a separation has not been observed (Murfet, 1973a). However, when further results are obtained the model may well need to be modified since there are several areas in which little direct evidence is at present available. Most important amongst these is the ratio of promotor to inhibitor and its relation to the threshold over the very early stages of growth (until the time node 10 is laid down). Whether seeds of different genotypes possess different ratios of the hormones at the start of germination is not clear. However, seeds which have received vernalising temperatures during their development flower substantially earlier than unvernalised seed indicating that hormonal differences may occur in seeds at the start of germination under some conditions. It has not been possible to show a similar carry-over effect between seeds (of the same weight) matured in long and short photoperiods (Reid and Murfet, 1974a). The sharpness of the lower cut-off limits for plants possessing different alleles at the *lf* locus suggests that if as suggested the thresholds remain unchanged during the growth of a plant the ratio of promotor to inhibitor must rise very rapidly over the first 2 weeks of growth, otherwise developmental noise, which clearly exists (e.g. L61a), would cause more variation in the flowering node amongst plants which flower below node 12.

The ratio of promotor to inhibitor appears to be of major importance in determining at least two other developmental processes as well as the change from the vegetative to the reproductive state. Firstly, it seems to control the development of the flower buds once they have been initiated, high ratios of promotor to inhibitor promoting development (Murfet, 1971a). In the present work this could be seen most

clearly in L61a plants under SD conditions where the first flower buds of impenetrant plants rarely developed into open flowers but those of penetrant plants did. This can readily be explained if it is the ratio of hormones present at the time the flower reaches a particular stage in its development which determines whether further development or abortion will occur. Consequently flower buds initiated at node 12-14 would be developing rapidly when a total of 16-22 nodes were present. At this stage in L61a plants the ratio of promotor to inhibitor is quite low and consequently abortion occurs (fig. 9.2a). However, at the time flowers are initiated in penetrant plants the ratio of promotor to inhibitor is rising rapidly and consequently full development of the flower buds occurs. There does not appear to be a particular threshold of the flowering hormones required for flower development, just a continuous increase in the amount of development as the ratio of promotor to inhibitor increases. Secondly, the ratio of promotor to inhibitor plays a significant role in the senescence of the plant. Whether this is purely an indirect effect caused by the control which the ratio exerts over floral development and consequently seed production, is not clear. It is well established that removal of the open flowers or young pods will substantially delay senescence of the apex (Lockhart and Gottschall, 1961) but whether this is due to the metabolic drain the reproductive organs place on the plant or the production by them of hormones which control the senescence of the plant is still debated. However, senescence of the apex will still occur in ED cultivars even if the flower buds are continually removed at an early age. It therefore seems possible that the ratio of the flowering hormones are directly involved in this process. Marx (1968, 1969) has reported a line of peas

(G2) which flowers at an early node but when grown under a short photoperiod develops over 50 reproductive nodes and produces a large number of seeds throughout this period. Murfet and Marx (1976) have suggested that this line has a flowering genotype of *lf E Sn Hr* and consequently it would be expected to possess a fairly inhibitory ratio of the flowering hormones from at least node 20 onwards. This inhibitory level of the flowering hormones could be the cause of the extended reproductive growth and this would be supported by the fact that an extension of the photoperiod with light that would increase the ratio of promotor to inhibitor has been shown to enhance senescence of this line (Proebsting and Davies, 1975). These results raise the question of the relationship between programs designed to study the flowering and the senescence of peas. It would seem essential for both characteristics to be studied in any such programs, the work of Marx (1968, 1969) and Murfet (1971a) showing the value of this approach.

It has not been determined whether the thresholds for flower initiation determined by the alleles at the *lf* locus also influence floral development. If they do not it might be expected that floral development may be more rapid in the sequence lf^a, *lf*, *Lf* and Lf^d. This does appear to occur when L53 *(lf e Sn hr)* and L24 *(Lf e Sn hr)* are compared under a long photoperiod but will not be fully answered until a scale to measure the rate of floral development is devised, many other genotypes are examined (eg. lf^a *e sn hr*, *lf e sn hr* and *Lf e sn hr)* and the effect of plant age on the ratio of promotor to inhibitor taken into account.

The relationship of specific points in this model to the models put forward by other workers has been examined in detail

in the relevant experimental chapters. However, a few generalised comments on the major differences of the present model from that of the other major groups studying the flowering of peas would seem warranted. Haupt (1969) and Köhler (1965) suggest late cultivars are normally autonomously determined (determination solely by aging) while early cultivars are normally induced by florigen contained in their own cotyledons. Late cultivars may be induced by grafting to an early stock while early cultivars may be forced to flower autonomously if their cotyledons are removed at an early stage. However, since induced late cultivars always flower later than induced early cultivars and autonomously determined early cultivars flower earlier than similarly treated late cultivars it was suggested that differences between the types also occurred in the ability of their apical meristems to become determined. The present model is basically similar to this with the exception that for reasons mentioned previously an inhibitor as well as a promotor is postulated. The autonomous flowering of late cultivars is similar to the suggested reduction in inhibitor production by *Sn* as the plant ages. The induction of early cultivars by florigen contained in their own cotyledons is similar to the promotory ratio of hormones shown to be produced by the cotyledons of both ED and EI cultivars (Murfet, 1971c, 1973b, Reid and Murfet, 1975a). The most significant difference is possibly the suggestion that cotyledon removal from the early cultivar Kleine Rheinländerin leads to autonomous determination of flowering. This may be expected if this variety is an EI type but Reid and Murfet (1975a) have shown it to be an ED type (possibly of genotype *lf sn hr*) and would expect it to be induced by a promotory level of the flowering hormones produced in the young shoot. The large delay observed by Köhler (1965) could be due to the poor growth rate expected

under the conditions used, although undoubtedly part of the response is attributable to inhibitor production by *sn*. The differing abilities of the apical meristems to be determined is what might be expected if the two cultivars used, Alderman and Kleine Rheinländerin, possessed the alleles *Lf* and *lf* respectively as has been suggested by Murfet (1976). This last point illustrates clearly how a full understanding of the genetics of a process can lead to a much clearer understanding of the physiology.

Barber (1959) suggested the gene *Sn* produced a flower inhibitor which was competitively destroyed by vernalisation and long days. Varieties carrying *sn* were thought to flower in the early region because they possessed no inhibitor, the question of a promotor being left unanswered. A model was however presented which suggested a promotor occurred and that it was the precursor of the inhibitor. These conclusions have several distinct differences from those indicated by the present results. Evidence has been produced to show that both an inhibitor and promotor exist and that light operates by influencing the production of inhibitor, not its breakdown. Vernalisation has been shown to have at least 2 modes of action, one through the ratio of promotor to inhibitor produced during the low temperature and a second that influences occurrences in the shoot, possibly the threshold of hormones required at the apex for flowering and/or the aging process. The present work has shown no need to postulate a connection between the promotor and inhibitor at the biochemical level and therefore supports the evidence brought forward by Murfet (1971c) against such a system. The work of Paton (1967, 1968, 1969, 1971), Amos and Crowden (1969) and Sprent (1966a, 1966b, and 1967) largely extends the work of Barber in specific areas and is not discussed in detail here since it has been discussed

in the relevant experimental chapters. However, the concept of a definite leaf requirement for flowering in late pea cultivars deserves further comment (Paton, 1967, 1968). This concept is analogous in some respects to the autonomous flowering of late cultivars proposed by Haupt (1969) and Köhler (1965) and to the phasing out of *Sn* activity as each plant ages as proposed by Murfet (1971b). This concept should therefore be considered as an alternative to the hypotheses of Haupt and Murfet and not as a separate mechanism affecting flowering. Using this approach the experimental results of Paton have been interpreted using the present model in the discussion in chapters 3, 4 and 5.

The last major theory in the literature for the control of flowering in peas is that proposed by Murfet (1971a, 1971b, 1971c, 1973a, 1973b, 1975). This theory has been clearly explained in chapter 1 and was used as the basis of the present work. During the course of the present study this theory has been extended substantially to include specific information on the control of inhibitor production by light, the effects of temperature and aging on the flowering process and the possible involvment of plant growth substances in this process. These results have either confirmed, or at least not been at variance with, the model proposed by Murfet.

Although comparison of the model developed for flowering in peas with those from other species may not be of great relevance due to the probable separate evolution of the control of flowering in different plant groups it would seem necessary since the results for other species may indicate the limits through which evolution has operated and place the results from peas in perspective. Amongst other LD plants, I know of no example where the

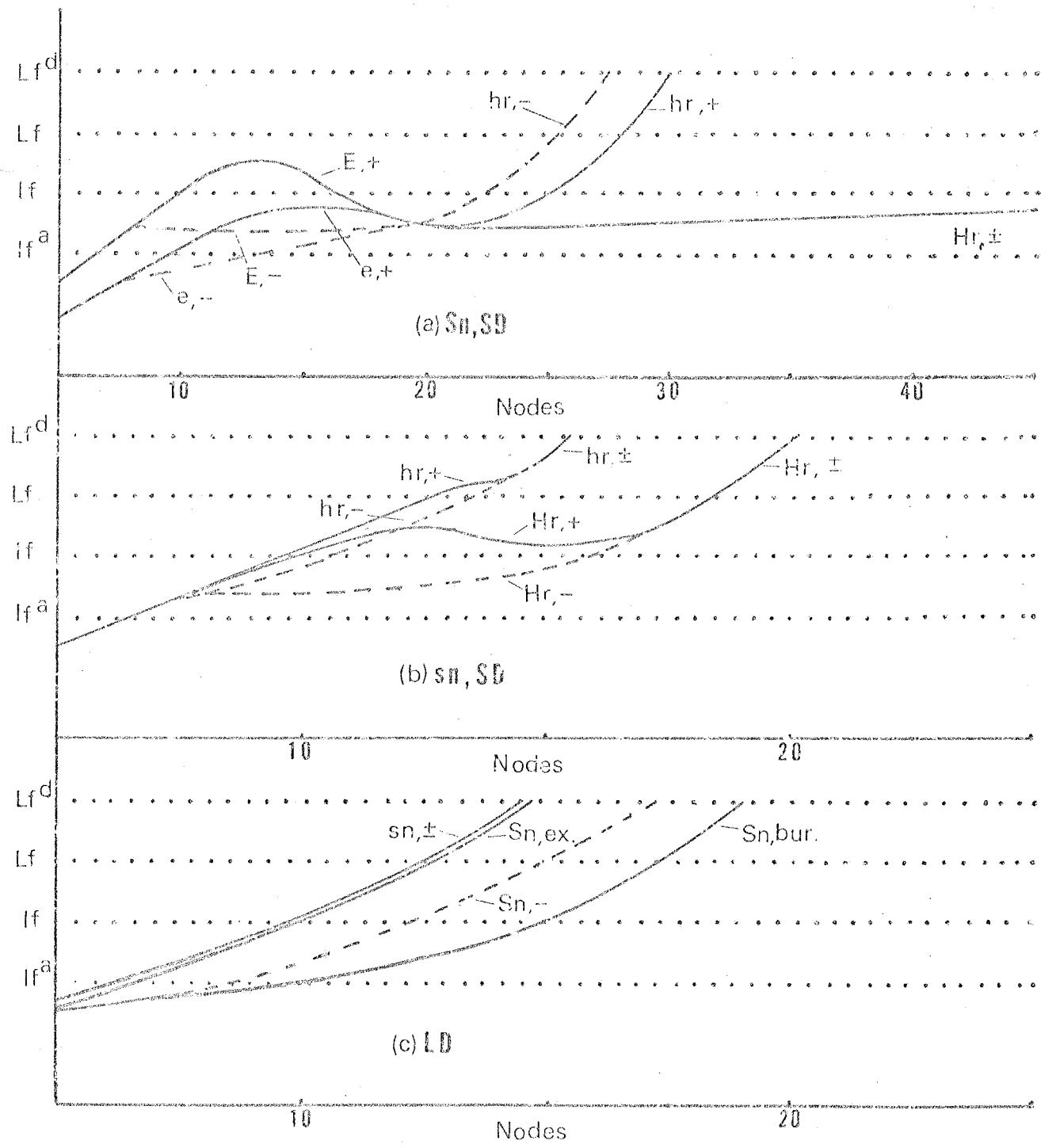
genetics of flowering and the action of photoperiod, vernalisation and aging have been studied in a single program although the genetics of the photoperiod and vernalisation responses have been studied in *Lolium*, *Hyocyamus niger*, *Secale* (Waring and Phillips, 1970), *Lunaria* (Wellensiek, 1973a), *Arabidopsis thaliana* (Napp-Zinn, 1962, 1963), *Silene armeria* (Wellensiek, 1969b), *Triticum aestivum* (Keim et al, 1973), etc. Several workers have presented general models of the control of flowering in LD plants (e.g. Chailachjan, 1937; Borthwick et al, 1948; Lang, 1965) but these will not be considered in detail as they are based on information from several different species with no concerted attempt to show that such a grouping of information is valid. Few comprehensive models concerning individual LD species have been put forward, although notable exceptions are *Lolium temulentum* (Evans, 1969) and *Silene armeria* (Wellensiek, 1969b). In both cases the models suggest that inhibition occurs in SD, although only in the case of *Lolium* does this appear to be a translocated hormone. At the apex in *Lolium* it is suggested the inhibitor and a promotor, which is formed in leaves exposed to photoperiods greater than the critical length, interact quantitatively to determine initiation. In *Silene*, long days and both high and low temperatures can overcome the inhibitory effects of SD and cause initiation by allowing the production of a flowering promotor. The varieties of *Lolium temulentum* used most frequently do not respond to vernalisation although winter annual types can respond. These few comments illustrate that parts of the models in both plants are similar to that in peas but in both cases distinct differences occur.

In most SD plants work suggests that leaves possess both an active promotory and inhibitory function (e.g. *Pharbitis nil* (Takimoto, 1969), *Glycine max* (Hamner, 1969), *Fragaria* (Guttridge, 1969), *Xanthium strumarium* (Salisbury, 1969)) although the nature of the inhibitory effects does not always appear hormonal. Again the control does not appear to be solely through the production of a flower promotor illustrating the model presented for peas is by no means unique.

Finally, some of the most similar responses to those reported in peas are found in other members of the *Leguminosae*. In *Vicia faba* both day neutral and quantitative LD types are found, the day neutral types flowering at the lowest node (Evans, 1959a). Low temperatures reduce the flowering node in all but the early, day-neutral types and become progressively more effective as the plants age. High temperatures (above 23^{o}C) inhibit flowering, and the production of an inhibitor under these conditions has been suggested. Considerable genetic variation is present within the group as the flowering node varies from 10 to 70 under the SD conditions used by Evans in his study of 4 commercial cultivars. In *Trifolium subterraneum* considerable genetic variation occurs in the flowering time and appears to be controlled by additive polygenes (Davern, Peak and Morley, 1957). The strains examined by Evans (1959b) behaved as quantitative long-day plants, although vernalisation was able to completely overcome the requirement for long-days. Evans suggests three interacting processes control flowering in *Trifolium subterraneum* firstly an inhibitory process which occurs only during the diurnal dark period, secondly a promotive process occurring at low temperatures and thirdly a promotive process occurring at high temperatures in continuous light.

The first of these is very similar to the action of the *Sn* gene in peas, while the third is similar to the response observed by Paton (1968) in the late pea cultivar Greenfeast and possibly occurs due to increased promotor production as the temperature is increased, inhibitor production not occurring due to the continuous light. The second process is similar to the effect of low temperatures reported in chapter 4. The above results are remarkably similar to those reported in peas but some differences have also been reported. For example, GA_3 promotes flowering in some long day cultivars of *Trifolium subterraneum* under warm SD conditions (Evans, 1959b), but delays it in long day cultivars of peas grown in similar conditions (chapter 8). The genetic variability shown in the above two species and the fact that the late habit is dominant to the early habit in both the sweet pea *(Lathyrus odoratus)* (Little and Kantor, 1941) and the garden bean(*Phaseolus vulgaris)* (Yarnel, 1965) show that peas are by no means unusual in the *Leguminosae* in possessing a large amount of genetic variability in the control of flowering. It seems likely that the mechanisms which control the flowering process in peas may well be a useful guide in examining the control of flowering in other species of this family, due to the already apparent similarities in their physiological behaviour.

Fig. 9.1 The proposed variation in the ratio of promotor to inhibitor with age is shown for various genotypes at the *e*, *sn* and *hr* loci exposed to either an 8h photoperiod (SD) or continuous light (LD). Arbitary thresholds controlled by alleles at the *lf* locus are shown. The effect of cotyledon removal on day 5 (-) and exposure of the cotyledons to the photoperiod from the start of germination (ex) are indicated where relevant, as well as intact plants (+) and plants with their cotyledons buried (bur.). A mean temperature of 20^{o}C is assumed.


Lf^d
Lf
lf
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hr,−
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E,+
E,−
e,+
e,−
Hr,±
(a) Sn, SD
10
20
30
40
Nodes
Lf^d
Lf
lf
lf^a
hr,±
hr,+
Hr,±
hr,−
Hr,+
Hr,−
(b) sn, SD
10
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Nodes
Lf^d
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lf
lf^a
sn,±
Sn,ex.
Sn,bur.
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(c) LD
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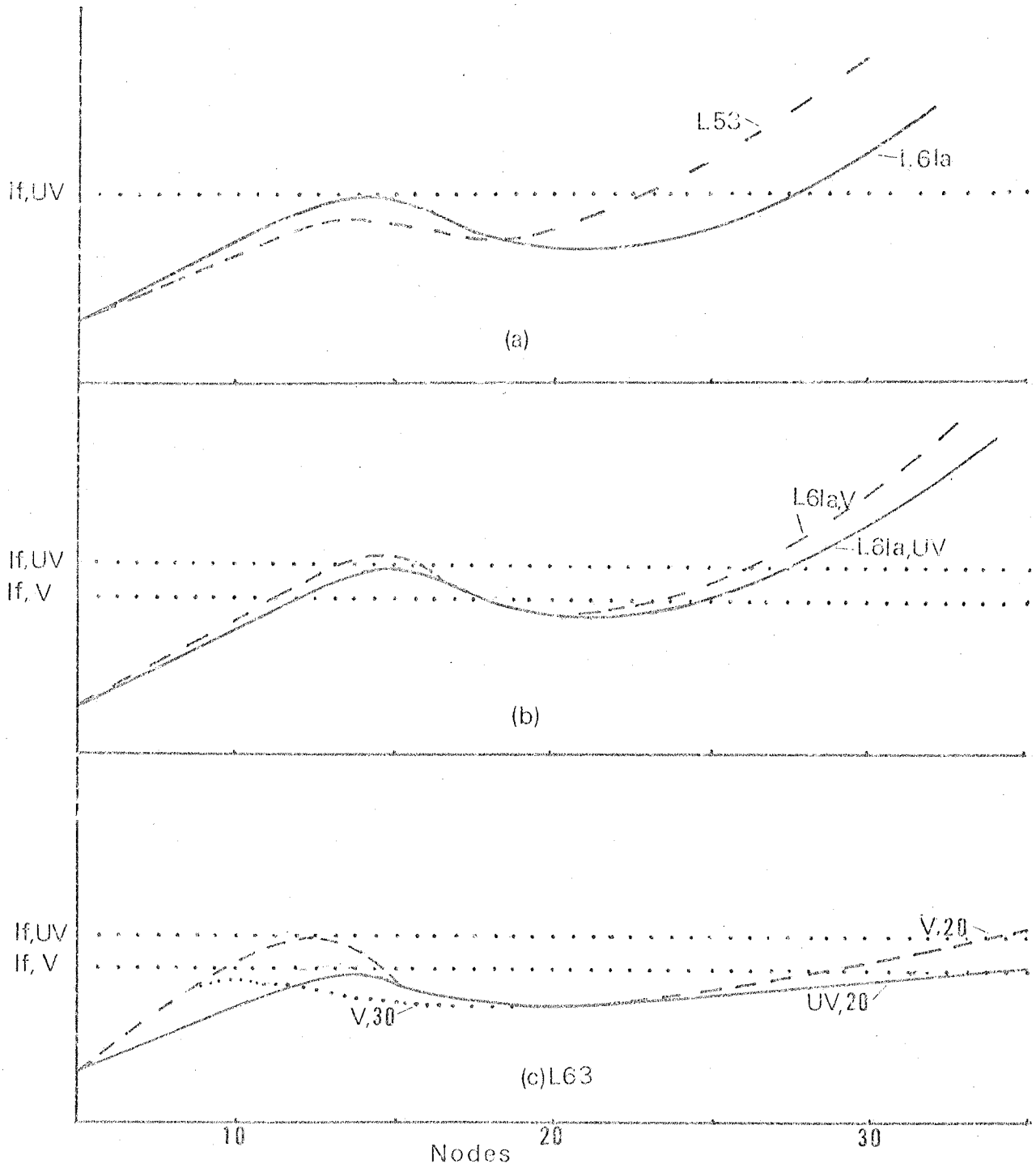
Nodes


L.53
L.61a
If,UV
(a)
L61a,V
L.61a,UV
If,UV
If, V
(b)
V,20
If,UV
If, V
V,30
UV,20
(c)L63
10
20
30
Nodes

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APPENDIX I

Appendix I contains the raw data relating to several experiments reported in the experimental chapters. A list of the tables and figures contained in this appendix is given below as well as references to the tables and figures in the experimental chapters derived from this raw data.

TABLES OR FIGS. IN APPENDIX I	RELEVANT TABLE IN THE TEXT.	CONTENT OF TABLES AND FIGS. IN APPENDIX I.
Table a	Table 4.3	Duration of Vernalisation
Table b	Table 4.6	Effect of Post-vernalisation temperature.
Fig. a	Fig. 3.1	Effect of age on sensitivity to long-day cycles.
Fig. b	Table 8.4	Response of L64 *(lf e sn Hr)* to GA_3, AMO1618 and CCC.

Table a Distribution of the node of first initiated flower for L63 *(If e Sn Hr)* treated as follows: decotyledonised when the plumules were about 2cm long and given either 1,2,3,4 or 5 weeks vernalisation from either the start of germination or after 24 days growth (leaf 5 expanded) or left unvernalised (UV)(notation 2 weeks vernalisation from day 0 is 2VO and 4 weeks vernalisation after leaf 5 has expanded is 4V5). The photoperiod was 8h.

	NODE OF FIRST FLOWER																						
TREATMENT	18 19	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	54	58	62	66	70+
5VO	-	7	6	5	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4VO	2	1	1	1	1	3	-	2	2	-	2	1	-	-	-	-	-	-	-	-	-	-
3VO	-	-	1	-	1	3	3	-	4	-	-	1	1	1	1	-	1	-	1	2	-	-
2VO	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	11	2	2	-	-
1VO	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	1	-	6	3	3	2
4V5	-	-	-	1	2	5	1	2	1	1	1	-	-	-	-	1	1	-	1	-	-	-
3V5	-	-	-	1	1	3	3	4	4	-	-	-	-	-	1	-	-	1	-	-	-	-
2V5	-	-	-	-	-	1	1	3	2	-	1	-	-	-	-	1	-	4	3	2	-	-
1V5	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	1	2	2	5	2	-
UV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	5	3	4	3	-	1

Table b. Distribution of the node of first initiated flower for L63 *(If e Sn Hr)* plants given either 4 weeks vernalisation (V) or no vernalisation (UV), followed by 2 weeks at a temperature of either 10, 15, 20, 25 or 31°C. After this treatment all plants were given SD conditions on the trucks. An 8h photoperiod was used throughout the experiment.

Treatment	Node of first flower																													
	11	12	13	14	15	16	17	18	19	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	54	58	62	66
UV 10	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2	2	2	1	1	3	4	-	-	-
V 10	9	12	2	-	2	1	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UV 15	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	2	2	1	3	1	1
V 15	9	13	3	1	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UV 20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	5	2	2	3	-
V 20	4	9	7	4	1	-	-	-	-	-	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UV 25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	2	-	1	-	4	2	2	-
V 25	1	2	-	6	1	-	-	-	-	-	1	1	2	4	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-
UV 31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	2	3	-	4	-
V 31	-	1	-	-	-	-	-	-	-	-	1	3	2	-	2	1	2	2	-	1	3	-	-	-	-	-	4	1	-

Fig. a The effect of age on the number of long day cycles (first cycle 32h, then multiples of 24h) required to cause 100 per cent flowering in lines 53 *(lf e Sn hr)*, 63 *(lf e Sn Hr)* and 24 *(Lf e Sn hr)*. The results for lines 53 (●) and 24 (□) came from one experiment, as well as some results for L63 (○). The results for L63 (▽) used to give the graph came from a separate experiment. All plants were grown in SD conditions on the trucks before and after treatment.

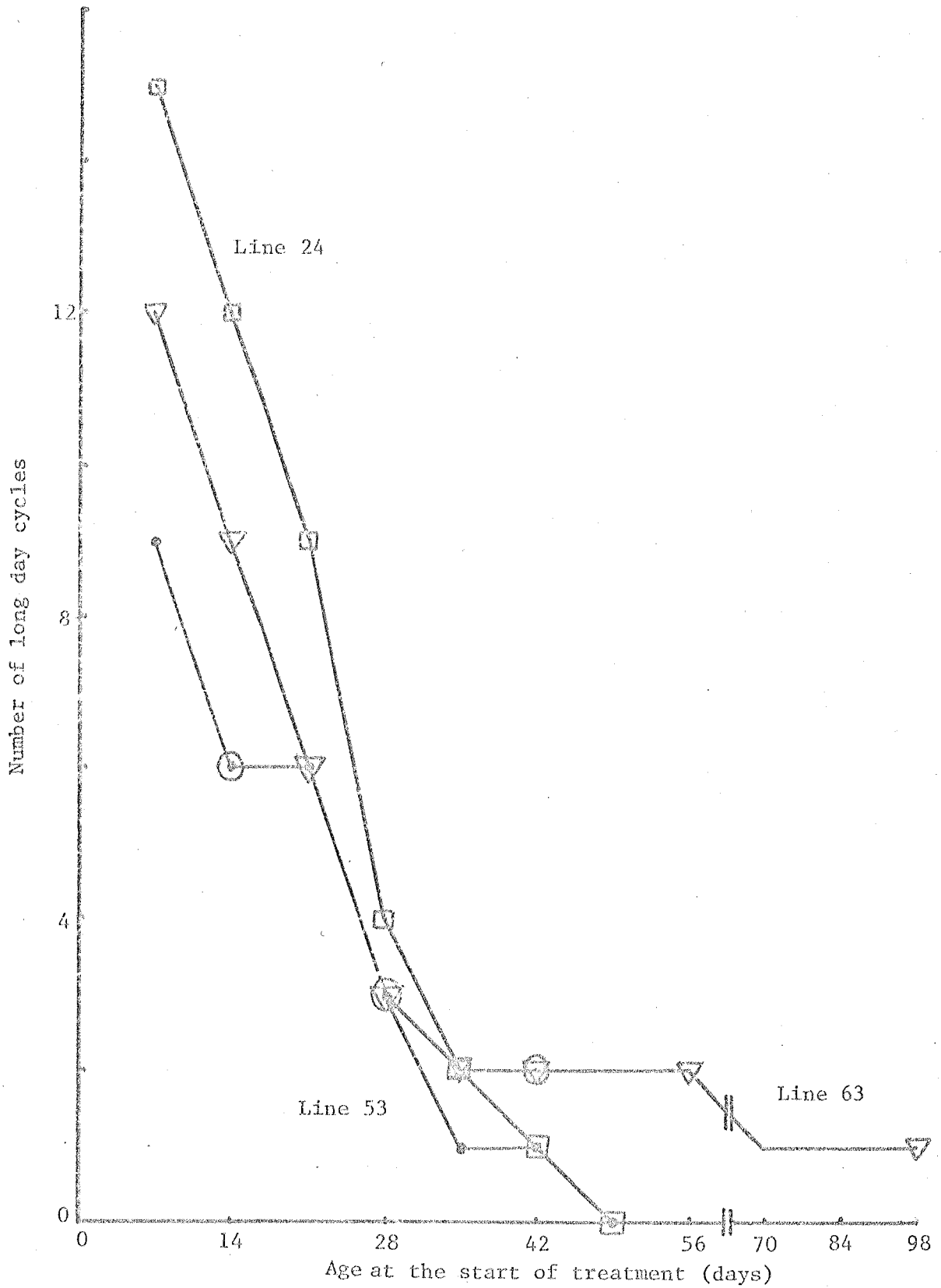
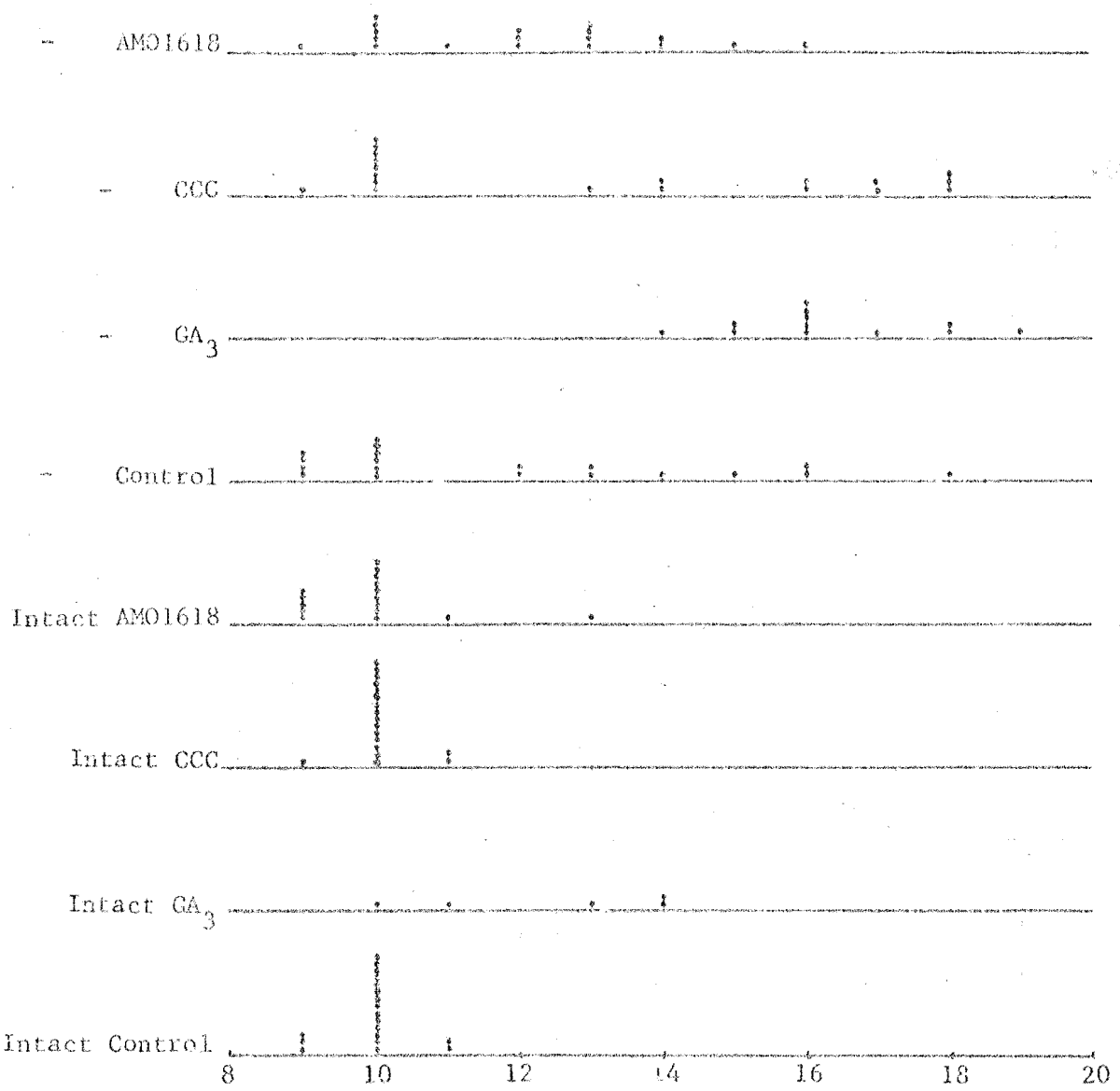

Line 24
Line 53
Line 63
Number of long day cycles
0
4
8
12
0
14
28
42
56
70
84
98
Age at the start of treatment (days)

Fig. b Distribution of the node of first initiated flower for intact or decotyledonised L64 plants *(lf e sn Hr)* either untreated (control) or treated with 10 µg of GA_3, 100 µg of AM01618 or 1000 µg of CCC. The photoperiod was 8h. The means are contained in table 8.4.


AM01618
CCC
GA3
Control
Intact AM01618
Intact CCC
Intact GA3
Intact Control
8
10
12
14
16
18
20

APPENDIX II

Appendix II contains a list of the work published by the author in conjunction with other workers.

Murfet, I.C. and Reid, J.B. (1973). Flowering in *Pisum*: evidence that gene *Sn* controls a graft-transmissible inhibitor. Aust. J. biol. Sci., 26, 675-677.

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Reid, J.B. and Murfet, I.C. (1974a). Effect of seed weight on flowering. *Pisum* Newsl., 6, 44-45.

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Reid, J.B. and Murfet, I.C. (1975b). Flowering in *Pisum*: the sites and possible mechanisms of the vernalisation response. J. exp. Bot., 26, 860-867.

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Wall, B., Reid, J.B. and Murfet, I.C. (1974). Differential response to cotyledon removal and vernalisation in early varieties. *Pisum* Newsl., 6, 50-51.

THE REGULATION OF FLOWERING IN *PISUM*.

JAMES B. REID

This booklet contains an extra copy of the tables and figures.

Table 2.1 The phenotypes and genotypes at four loci controlling flowering are shown for the lines used during the present study.

LINE NUMBER	PHENOTYPE	GENOTYPE
7	VEI	*lf*^{a} *E Sn hr*
8	EI	*lf E Sn hr*
24	L	*Lf e Sn hr*
51y	ED	*lf E sn Hr**
53	L	*lf e Sn hr*
58	ED	*lf e sn hr*
59	ED	*lf E sn hr*
60	EI	*lf E Sn hr*
61a	EI/L	*lf e Sn hr*+
63	LHR	*lf e Sn Hr*
64	ED ∅	*lf e sn Hr*
68	ED	*lf e sn hr*

*This line may be heterogeneous at the *E* locus.

+This line possesses a polygenic background which lowers the penetrance of *Sn* to approximately 0.5 under normal SD conditions.

∅ This line possesses distinct EI tendencies.

Fig. 2.1 Regression of total nodes present plotted against time ($y = 0.50x + 6.11$) and the number of leaves expanded against time ($y = 0.42x - 1.38$) for L63 plants exposed to an 8h photoperiod on the trucks. Both regressions are significant at the 0.001 level.

Fig. 2.1

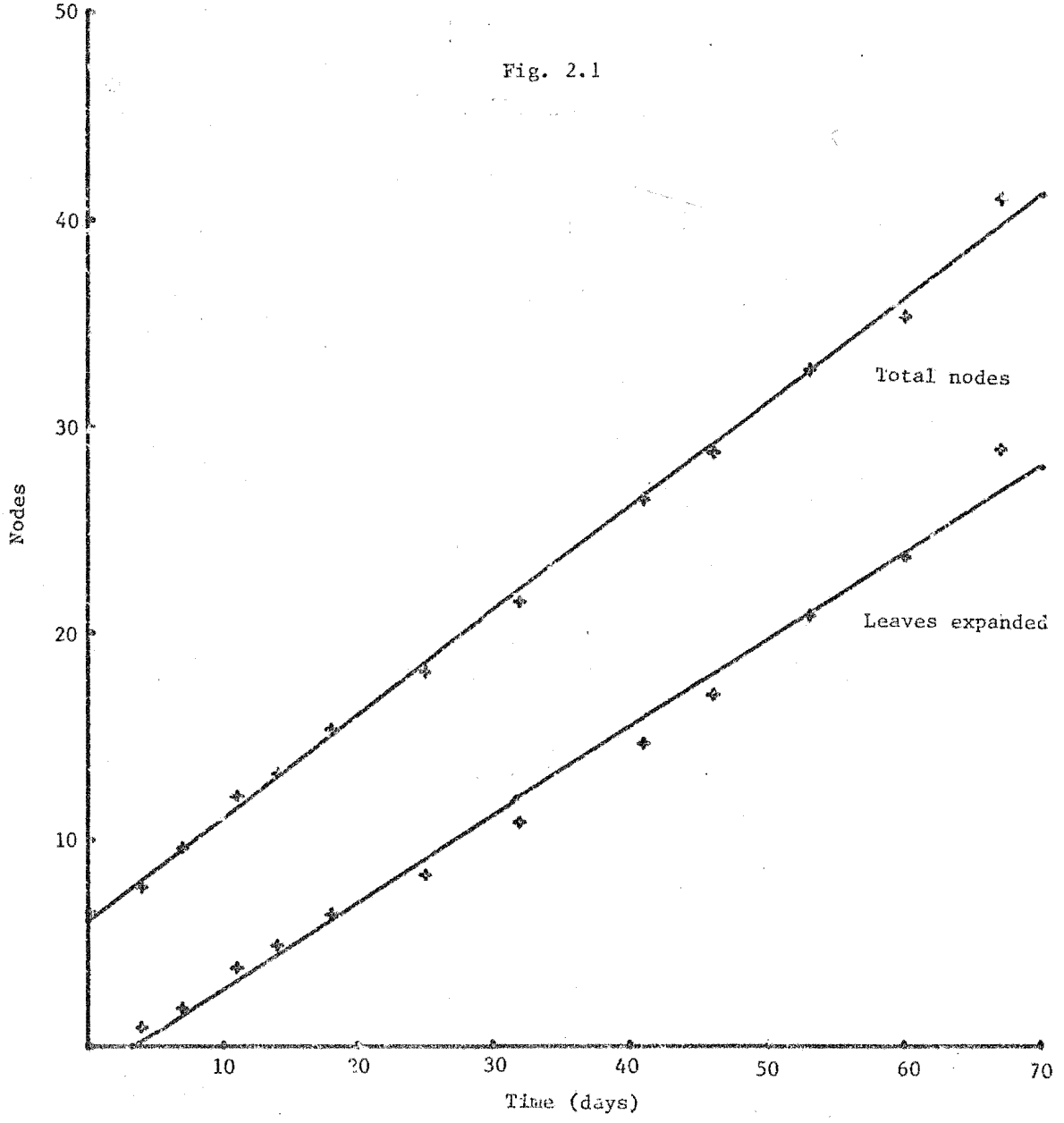

50
40
30
20
10
Nodes
Total nodes
Leaves expanded
10
20
30
40
50
60
70
Time (days)

Table 3.1 Mean node of first initiated flower ±S.E. for lines 58 *(lf e sn hr)*, 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)* given 8h of white light and 16h of weak incandescent light each day from the start of germination.

L58		L53		L63	
$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
13.52 ± .37	19	13.30 ± .36	20	12.26 ± .37	19

Table 3.2 Mean node of first initiated flower ± S.E. for lines 58 *(lf e sn hr)* and 53 *(lf e Sn hr)* exposed to continuous light from the start of germination. Three separate batches of L53 seed were used.

L58		L53(1)		L53(2)		L53(3)	
$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
10.76 ± .16	17	12.28 ± .37	18	13.11 ± .32	18	12.78 ± .27	18

Table 3.3 The mean node of first initiated flower ±S.E. for lines 58 (*lf e sn hr*), 53 (*lf e Sn hr*) and 24 (*Lf e Sn hr*) with their cotyledons either exposed to the photoperiod from the start of germination (DO) or buried 3 cm below the surface of the growing medium (D∞). The plants were exposed to a photoperiod of either 18, 20 or 24h light.

Photoperiod	58,DO	53,DO	58,D∞	53,D∞	24,DO
24	12.00 ± .47	12.63 ± .46	10.53 ± .13	14.57 ± .17	17.38 ± .27
20	11.63 ± .44	14.19 ± .37	10.27 ± .12	15.47 ± .19	17.93 ± .23
18	12.27 ± .41	15.00 ± .35	10.19 ± .10	15.53 ± .22	19.61 ± .14

Table 3.4 Mean node of first initiated flower ±S.E. for lines 58 *(lf e sn hr)* and 53*(lf e Sn hr)* with their cotyledons either shaded or exposed to either an 8 or 18h photoperiod from day 5. The plants were either left intact, decotyledonised (-), grafted on day 5 (e.g. 58/53) or had leaf 4 shaded (L4Sh).

Photoperiod	18 hour				8 hour			
Cotyledons	Shaded		Exposed		Shaded		Exposed	
	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n
53,intact	14.29 ± .16	21	12.57 ± .24	21	21.00 ± .49	15	23.47 ± .70	15
58,intact	10.22 ± .22	23			10.17 ± .12	24		
58/53	12.47 ± .17	17	11.67 ± .16	15	14.94 ± .17	18	14.47 ± .19	15
58/58	11.47 ± .13	15	11.90 ± .23	10	10.89 ± .16	18	11.33 ± .17	9
53,-	12.27 ± .18	15			17.50 ± .49	14		
58,-	10.58 ± .15	24			10.38 ± .25	24		
53,L4Sh.	14.90 ± .14	20			22.28 ± .55	14		

Table 3.5 The percentage of red light to red plus far-red light in the light sources used during the present study.

SOURCE	% $\frac{\text{Red}}{\text{far-red + red}}$
RED	89.7
FAR-RED	$<$.02
FLUORESCENT TUBE	89.3
GRO-LUX TUBE	97.3
INCANDESCENT BULB	44.4
PHILIPS PF712 BULB	43.8
BLUE	Undetectable

Table 3.6 The percentage of plants induced to flower by the experimental treatment and the mean node of first initiated flower ± SE for L63 plants *(lf e Sn Hr)* exposed to an 8h natural photoperiod and then given various combinations of treatments with light from mixed incandescent-fluorescent sources (L), red light at an intensity of 20 $\mu W/cm^2$ (R), red light at an intensity of 50 $\mu W/cm^2$ (RH), far-red light (FR), blue light (B) or darkness (D) during the remaining 16h each day. The length of exposure each day to each treatment is indicated in hours preceding the treatment symbol. The age (in days) of the plants at the completion of the experimental treatment is also given.

EXPERIMENT	TREATMENT	% FLOWERING	FLOWERING NODE ($\bar{x}$±S.E.)	n	AGE (days)
1	16L	100	14.52±.25	21	56
1	16R	100	28.21±.50	19	56
1	16FR	100	18.18±.54	17	56
1	16B	0	42.89±.40	19	56
2	16L	100	14.83±.51	12	61
2	7D 2R 7D	100	23.63±.35	19	61
2	7D 2FR 7D	0	45.33±.88	18	61
2	16D	0	48.00±.96	15	61
3	16L	100	18.83±.17	12	52
3	7D 1R 8D	100	27.95±.46	19	52
3	8D 2FR 6D	25	49.92±2.24*	24	52
3	7D 1R 2FR 6D	100	31.60±.29	20	52
3	16D	0	57.13±2.83	8	52
4	16L	100	17.25±.13	4	41
4	8D 8R	100	21.10±.24	21	41
4	8FR 8D	29	41.00±2.00*	21	41
4	8FR 8R	100	19.95±.15	21	41
4	16D	0	44.83±1.02	12	41
5	16L	100	14.75±.41	8	45
5	8R 8D	11	45.95±1.52*	19	45
5	8D 8FR	58	33.67±2.17*	21	45
5	8R 8FR	11	45.37±1.43*	19	45
5	16D	0	52.83±1.25	6	45
6	16D	0	51.38±1.96	13	64
6	8FR 8D	53	39.41±1.69*	17	64
6	8D 8FR	100	25.71±.67	14	64
6	4D 8FR 4D	100	29.24±.79	17	64
7	16L	100	19.14±.34	7	63
7	7.75D .5R 7.75D	100	31.87±.32	23	63
7	7.75D .25R 8D	27	52.50±2.19*	22	63
7	7.75D .25RH 8D	100	32.75±.59	20	63
7	16D	0	63.18±1.08	11	63
8	8R 8D	85	38.70±2.30*	20	60
8	4R 2D 2R 8D	73	41.64±2.80*	22	60
8	2R 4D 2R 8D	100	31.67±.37	9	60
8	6D 2R 8D	100	26.63±.37	19	60
8	16D	0	64.22±2.96	9	60
8	16L	100	14.91±.16	11	60

* This flowering node is the mean for all plants given a particular treatment.

Table 3.7 The mean node of first initiated flower ±SE. for L63 plants exposed to an 8h photoperiod and then transferred each day to dark compartments and given either 16h of light from incandescent bulbs of 4 different intensities or 7.5h of darkness, 1h of red light at 4 different intensities followed by a further 7.5h of darkness till day 60.

INTENSITY ($\mu W/cm^2$) of INCANDESCENT	CONTINUOUS $\bar{x}$ ± S.E.	n	INTENSITY ($\mu W/cm^2$)	1 HOUR FLASH $\bar{x}$ ± S.E.	n
8,200	14.08±.25	24	56	25.55±.41	20
1,800	14.87±.17	23	20	25.23±.32	18
200	15.36±.36	22	5	24.47±.24	15
60	15.25±.36	24	1	27.67±.54	18

Table 3.8 The mean node of first initiated flower ± SE. for L63 plants exposed to an 8h photoperiod followed by either 16h of light or 7h darkness, 2h light, 7h darkness. The light was provided by either Philips PF712 bulbs, incandescent bulbs, fluorescent tubes or gro-lux tubes at the intensity specified. The age of the plants at the completion of treatment was 52 days.

LIGHT TYPE	INTENSITY	CONTINUOUS		2 HOUR FLASH	
		$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
RUBY-RED	800 $\mu W/cm^2$*	17.33±.19	21	23.33±.20	21
INCANDESCENT	800 $\mu W/cm^2$	15.65±.13	20	21.81±.29	16
FLUORESCENT	95 $\mu W/cm^2$	23.18±.20	17	24.48±.20	21
GRO-LUX	80 $\mu W/cm^2$	23.29±.19	17	25.16±.31	19

* In the continuous treatment the intensity was only 300 $\mu W/cm^2$.

Table 3.9 The percentage of plants induced to flower by the experimental treatment and the mean node of first initiated flower ± S.E. for L63 plants exposed to the varying cycles of white light (L) and darkness (D) indicated in the treatment column. The duration of each treatment is indicated in hours preceding the treatment symbol. The light was from a mixed incandescent-fluorescent source. The number of plants scored (n) and the age at the start and finish of the treatment is indicated.

Experiment	Treatment	Percent flowering	Flowering node $\bar{x}$ ± S.E.	n	Age (days) Start	Finish
9	12L 12D	15	45.31 ± 2.01	13	46	63
9	24L 24D	100	26.80 ± .49	5	46	63
9	36L 36D	100	26.88 ± .35	8	46	63
9	8L 16D	0	46.38 ± .78	8	46	63
10	12L 12D	81	41.50 ± 1.77	20	50	71
10	12L 24D	0	57.72 ± .99	18	50	71
10	12L 36D	0	53.77 ± 1.88	13	50	70
10	12L 48D	0	55.84 ± 1.24	19	50	70
10	12L 60D	0	51.11 ± 1.82	9	50	71
11	4L 6D 2L 12D	0	57.89 ± .87	19	29	45
11	4L 8D 2L 10D	0	59.29 ± 1.18	17	29	45
11	4L 10D 2L 8D	100	34.60 ± 1.05	20	29	45
11	8L 6D 2L 8D	100	28.00 ± .22	20	29	45

Table 3.10 The percentage of L63 plants induced to flower by either 1,2,3 or 4 LD cycles (first cycle 32h, then multiples of 24h) after exposure to cycles of varying lengths (indicated in hours) of light (L) and dark (D) from the time the shoot emerged until day 29. The light was from a mixed incandescent-fluorescent source. After exposure to the LD cycles the plants were transferred to SD conditions on the trucks.

TREATMENT	Number of LD Cycles							
	1		2		3		4	
	% flowering	n	% flowering	n	% flowering	n	%flowering	n
12L 36D	0	25	0	24	19	26	44	25
10L 12D 2L 24D	0	24	16	25	46	26	88	25
10L 24D 2L 12D	0	28	33	27	66	29	89	27
10L 30D 2L 6D	0	24	11	27	41	27	69	29

Fig. 3.1 The effect of age on the number of LD cycles (first cycle 32h of light, then multiples of 24h) required to induce 50 per cent (solid lines) and 100 per cent (broken lines) flowering in lines 53 *(If e Sn hr)*, 63 *(If e Sn Hr)* and 24 *(Lf e Sn hr)*. The points have been interpolated from the raw data of two separate experiments. The plants were grown in an 8h photoperiod on the trucks before and after treatment.

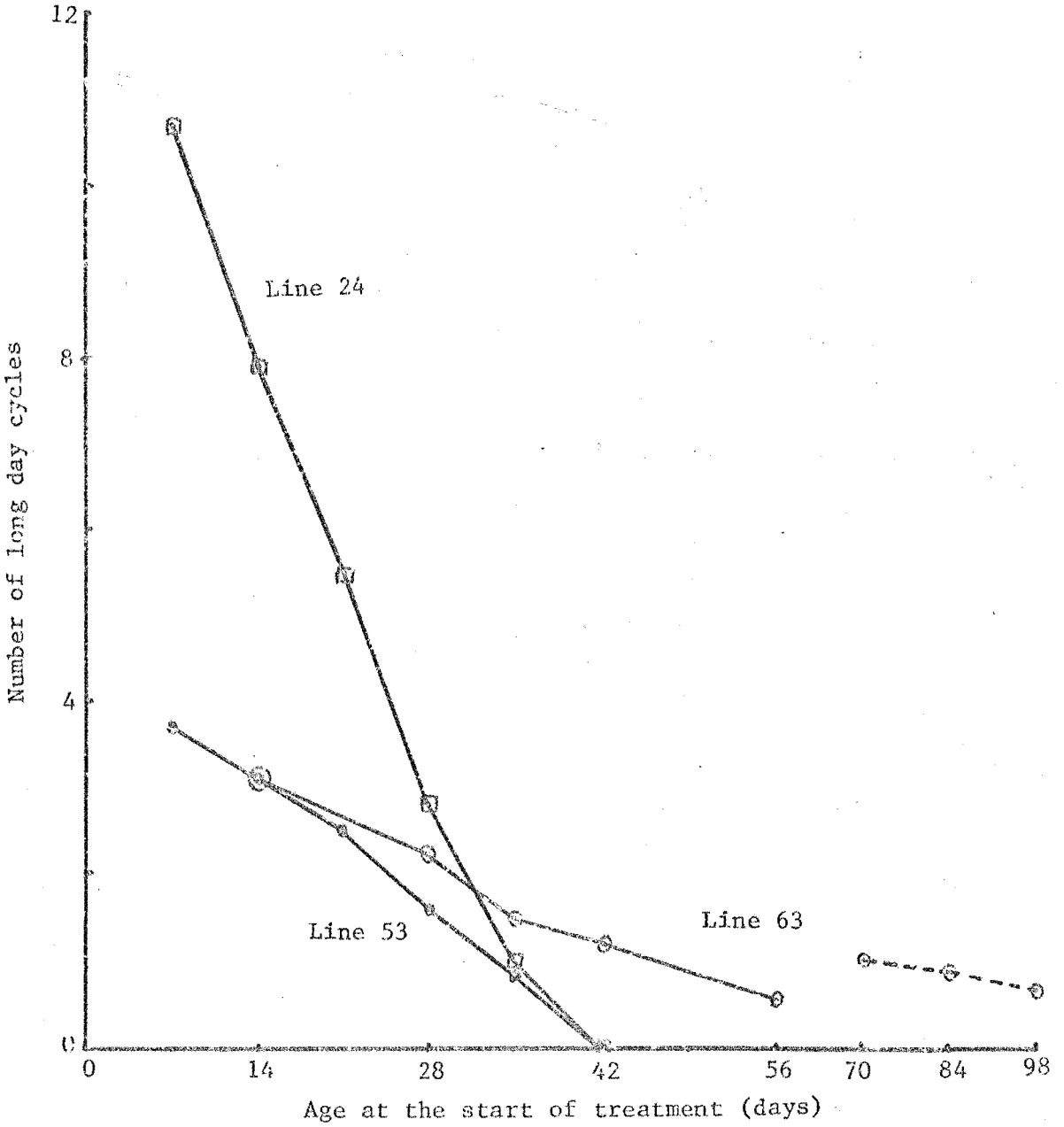

12
8
4
0
Number of long day cycles
Line 24
Line 53
Line 63
0
14
28
42
56
70
84
98
Age at the start of treatment (days)

Fig. 3.2 Mean node of first flower $\pm$S.E. for 163 plants exposed to 12, 13, 14, 15, 16, 18, 20 and 24h photoperiods from a mixed incandescent-fluorescent source at a temperature of 17.5°C. Under the 13h photoperiod only 8 plants (36%) were induced before the completion of treatment when 25.74$\pm$.23 leaves were expanded. The point for 13h on the graph comes only from these 8 plants. No plants were induced to flower in the present experiment by a 12h photoperiod. The minimum number of plants scored per treatment was 16.

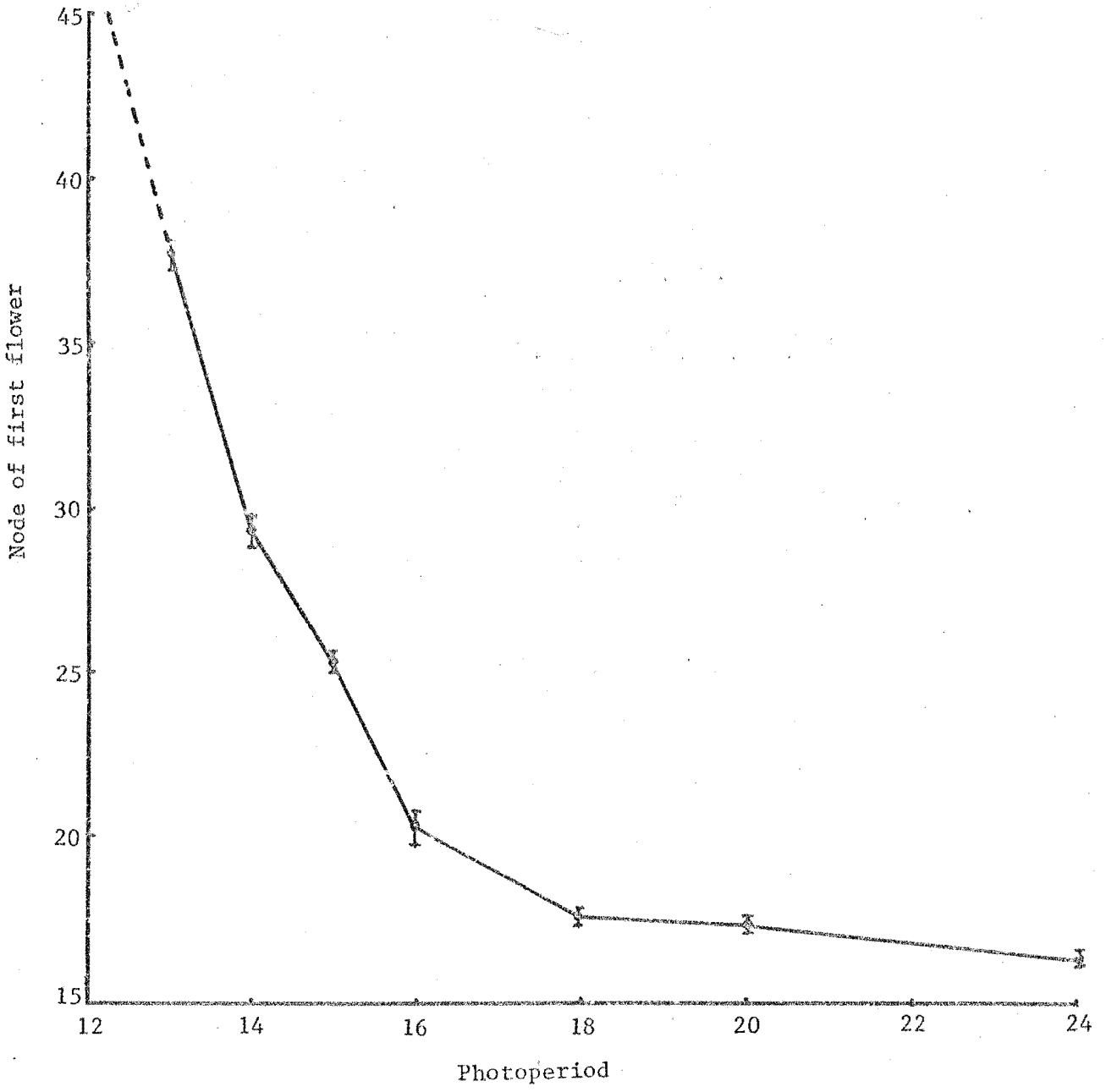

45
40
35
30
25
20
15
Node of first flower
12
14
16
18
20
22
24
Photoperiod

Fig. 3.3 Mean node of first initiated flower ± S.E. for plants of L53 *(lf e Sn hr)* and L58 *(lf e sn hr)* grown in a photoperiod of 18h (P18) or continuous light (P24) and given the following treatments: seed planted (buried) in the usual manner (C); the cotyledons and shoot exposed from day 6 (D6) or from the start of germination (DO); embryos excised from the cotyledons 18-27h from the start of imbibition, the appropriate photoperiod applying from the start (E). Eighteen plants were used per treatment.

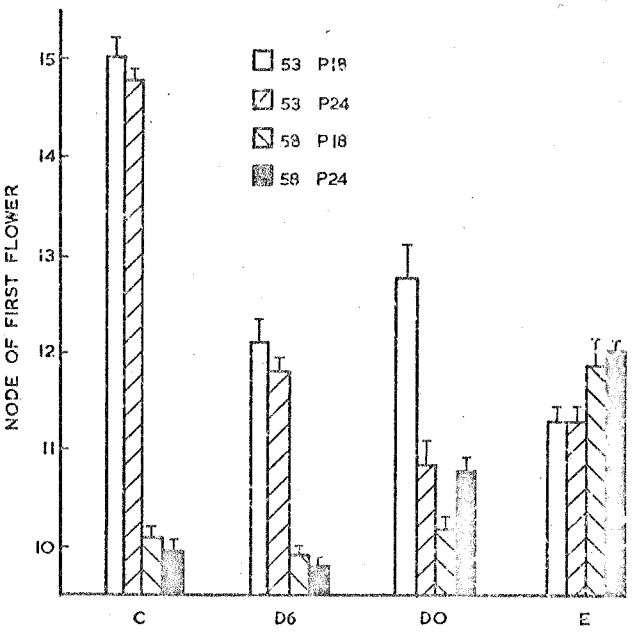

NODE OF FIRST FLOWER
15
14
13
12
11
10
53 P18
53 P24
58 P18
58 P24
C
D6
DO
E

Fig. 3.4 Regression of mean flowering node for the progeny of L53 plants *(lf e Sn hr)* plotted against the flowering node of the parent ($y = 0.21x + 8.32$). All plants were exposed to continuous light from the start of germination. The slope of the regression is significantly different from 0 (at the 0.05 level).

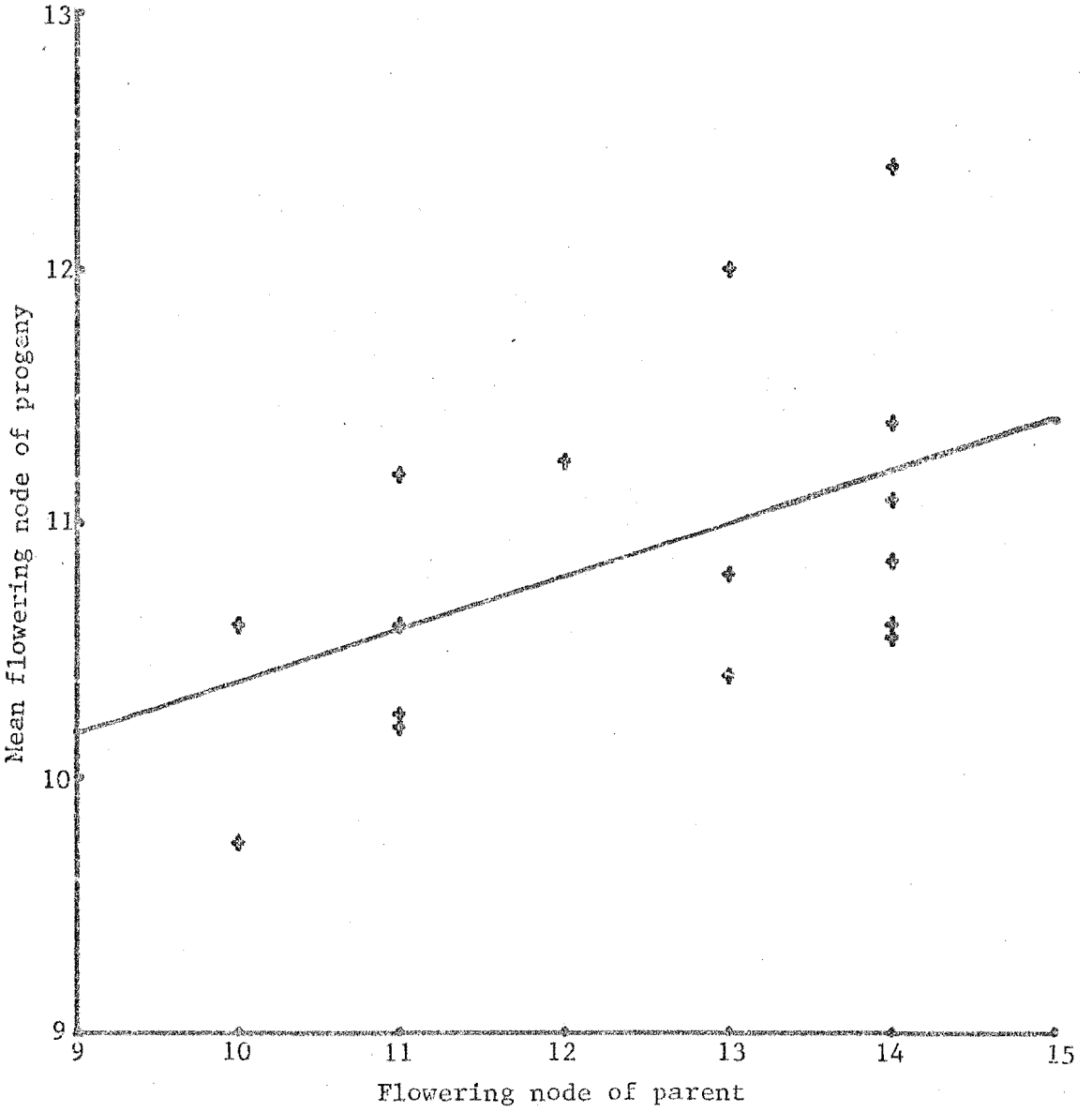

13
12
11
10
9
Mean flowering node of progeny
9
10
11
12
13
14
15
Flowering node of parent

Fig. 3.5 Regression of mean flowering node of progeny of L53 plants *(lf e Sn hr)* plotted against the flowering node of the parent ($y = 1.15x + 10.38$). The parental plants were exposed to continuous light from the start of germination while the progenies were exposed to an 8h photoperiod. The slope of the regression is significantly different from 0 (at the 0.01 level).

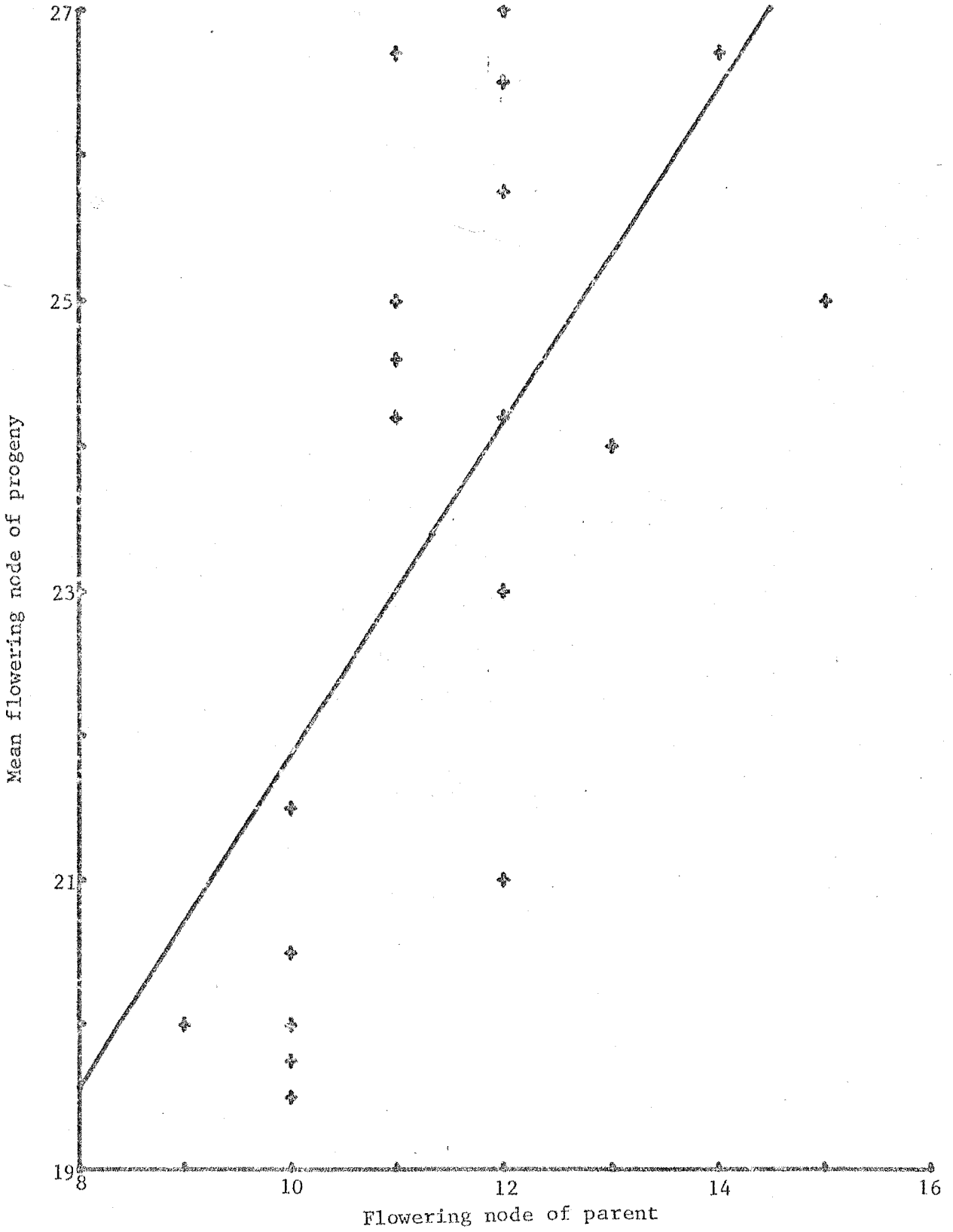

27
25
23
21
19
Mean flowering node of progeny
8
10
12
14
16
Flowering node of parent

Fig. 3.6 The mean node of first initiated $\pm$ S.E. for L63 plants *(lf e Sn Hr)* exposed to an 8h photoperiod followed by a 16h night interrupted by 2h of red light after either 4,5,6,8 or 10h darkness. The plants exposed to 4h darkness prior to treatment with red light did not initiate until after transfer to a long photoperiod.

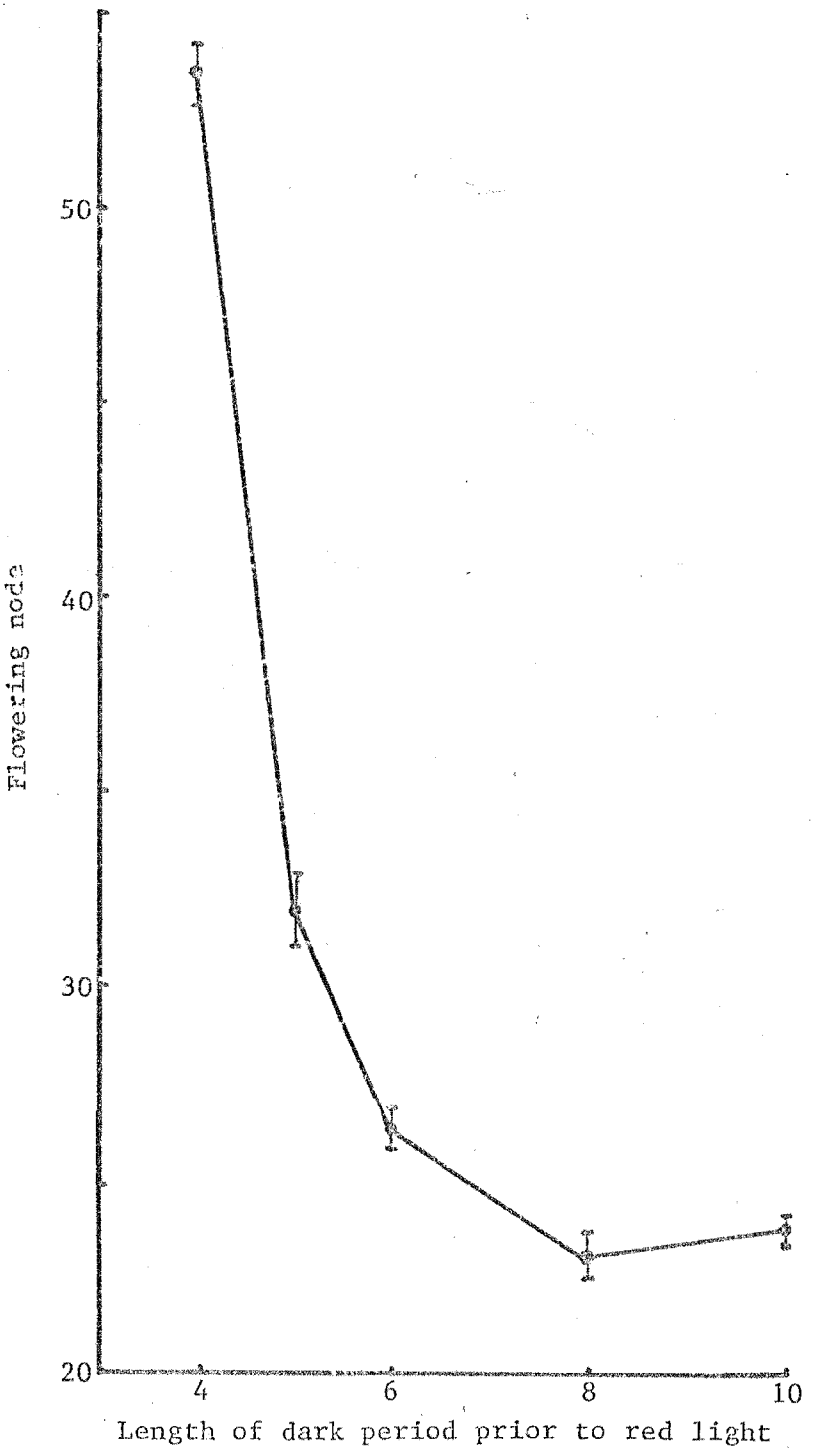

Flowering node
50
40
30
20
4
6
8
10
Length of dark period prior to red light

Table 4.1 Distribution of the node of first initiated flower for L63 treated as follows: unvernalised (UV) and vernalised (V) intact plants, unvernalised and vernalised decotyledonised plants (UV- and V- respectively) and grafted in various ways (e.g. unvernalised scion and stock UV/UV). The numbers in brackets represent slow grafts. Grafts and cotyledon removal were performed when the epicotyl reached a length of 1-2 cm. The photoperiod was 8h.

Treatment	Node of first flower																					
	9 10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	49 50
UV	-	-	-	-	-	-	-	-	-	-	-	-	1	4	4	2	0	1	1	2	1
V	1	5	6	2	-	1	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
UV-	-	-	-	-	-	-	-	-	-	-	-	1	1	2	4	3	5	1	2	-	-
V-	-	-	-	-	1	18	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
UV/UV	-	-	-	-	-	-	-	-	-	-	-	2(1)	2	(2)	1(1)	0	1	3	2(1)	-	-
V/V	-	2	3	-	-	(2)	1	(1)	-	-	-	-	-	-	-	-	-	-	-	-	-
UV/V	-	2	5	1	-	-	-	-	-	-	1	0	(1)	2	0	0	0	0	0	1	-
V/UV	-	-	-	-	3(1)	1	5(3)	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Table 4.2 The number of L63 plants falling into the low or combined middle and high regions after being exposed to 0, 1, 2, 3 or 4 weeks vernalisation from the start of germination followed by SD conditions on the trucks.

Vernalisation (weeks)	Number of plants per class	
	Low	Middle and High
0	0	22
1	1	19
2	3	18
3	4	16
4	7	12

Table 4.3 The number of decotyledonised L63 plants falling into the middle and high regions after 0, 1, 2, 3, 4, or 5 weeks vernalisation either from the start of germination or after 24 days growth. The photoperiod was 8h. The cutoff between the classes was at node 40, the distribution of the flowering nodes being given in Appendix 1.

Vernalisation	From day 0		From day 24	
(weeks)	number of plants middle	high	number of plants middle	high
0	0	18		
1	0	18	1	14
2	0	18	8	10
3	12	8	16	2
4	15	1	14	3
5	19	0		

Table 4.4 Distribution of the node of first flower for L63 treated as follows:left unvernalised (UV) or vernalised for 4 weeks from either the start of germination (VI-4), after 4 days growth (V5-8), 8 days growth (V9-12) or 12 days growth (V13-16). The photoperiod was 8h.

Treatment	Node of first initiated flower																			
	10 11	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48 49
V1-4	1	10	3	2	-	-	2	2	2	3	5	2	2	-	-	-	-	2	-	-
V5-8	-	2	5	6	-	-	1	3	4	4	3	2	-	-	-	-	1	-	-	-
V9-12	-	-	-	-	1	-	-	2	6	8	9	2	-	1	-	-	-	-	-	-
V13-16	-	-	1	-	-	-	1	4	2	10	8	-	-	-	-	1	-	-	-	-
UV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	7	11	2

Table 4.5 Distribution of the node of first initiated flower for L63 plants given an 8h photoperiod either on the trucks at an average temperature of 17°C (17) or at a temperature of 3, 6, 9 or 12°C in growth cabinets for either the first 38 days of growth (3T, 6T, 9T and 12T respectively) or until the plumules were 2.5 to 3cm long (3D, 6D, 9D and 12D respectively). At the completion of these treatments all plants received an 8h photoperiod on the trucks.

Experiment	Treatment	Node of first flower																											
		11	12	13	14	15	16	17	18	19	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	49 50			
1	17	1	1	1	2	1	-	-	-	-	-	-	-	-	1	1	-	-	1	-	3	3	2	3	5	3			
1	12T	1	4	2	1	3	2	-	-	-	-	-	-	1	-	2	1	1	4	2	2	1	2	-	-	-			
1	9T	-	1	4	3	7	8	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
1	6T	1	4	6	6	9	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
1	3T	5	18	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2	12D	-	2	1	3	1	1	-	-	-	-	-	-	1	-	-	1	-	-	2	1	1	1	3	5	5			
2	9D	-	6	2	3	1	1	-	-	-	-	-	-	-	-	-	-	-	1	1	1	2	3	2	3	3			
2	6D	2	1	3	4	11	3	3	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
2	3D	9	18	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

Table 4.6 The number of plants falling into the low, middle and high regions for L63 plants given either 4 weeks vernalisation (V) or no vernalisation (UV) followed by 2 weeks at either 10, 15, 20, 25 or 31°C before transfer to normal temperatures on the trucks. Node 17 was used as the cutoff between the low and middle regions and node 38 between the middle and high regions (for distribution see Appendix 1). The photoperiod was 8h. The percentage of plants flowering in the low region which showed vegetative reversion and the average number of nodes of this reversion are indicated.

	Temperature	Number of Plants			% Reversion	Av. length of Reversion
	°C	Low	Middle	High		
UV	10	2	1	15	0	-
	15	1	0	13	0	-
	20	0	0	15	0	-
	25	0	0	13	0	-
	31	0	0	11	0	-
V	10	26	3	0	15	1.0
	15	26	3	0	46	2.2
	20	25	4	0	88	9.1
	25	10	12	0	100	9.8
	31	1	13	9	100	12.0

Table 4.7 Distribution of the node of first initiated flower in 2 experiments using L63 and an 8h photoperiod. In the first all plants received 33 days of vernalisation followed by SD conditions on the trucks which were either not interrupted (treatment C) or interrupted after 0, 1 or 2 weeks by 2 weeks at 32°C (designated treatments 0,1 and 2 respectively). The percentage of vegetative reversion amongst plants flowering in the low region (11-16) is indicated. In the second experiment all plants received 32 days of vernalisation followed in most by 9 days at 30°C in either complete dark (D), 8h of weak fluorescent light (L) or 8h of high intensity light from a mixed incandescent-fluorescent source (H) before transfer to an 8h photoperiod on the trucks. One treatment (V) was not exposed to the high temperature, being transferred to the trucks immediately after vernalisation.

		Node of first flower																									
Experiment	Treatment	11	12	13	14	15	16	17	18	19	20	22	24	26	28	30	32	34	36	38	40	42	44	45 46	Percent Reversion
1	C	2	9	6	4	1	2	-	-	2	-	1	-	-	1	-	-	-	-	-	-	-	-	-	0
1	O	1	2	3	-	3	1	-	1	-	2	8	5	2	-	-	-	-	1	-	-	-	-	-	100
1	1	2	2	11	7	1	-	-	-	-	1	1	1	-	-	-	1	-	-	-	-	-	-	-	86
1	2	3	5	5	9	2	1	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	100
2	V	1	2	3	4	1	1	1	1	1	1	5	2	2	1	-	-	-	-	-	-	-	-	-	-
2	H	3	3	2	-	-	-	-	-	1	2	2	3	1	3	4	-	-	-	-	-	-	-	-	-
2	L	2	1	1	-	-	-	-	-	-	1	1	8	1	1	3	2	-	-	-	-	1	1	-	-
2	D	-	-	1	1	-	-	-	-	-	-	-	-	5	4	1	3	2	2	1	-	-	-	1	-

Table 4.8 Distribution of the node of first initiated flower for L63 plants grown on the trucks from seeds that were exposed to 7.5°C or to normal growing temperatures (approx. 20°C) from the time of fertilisation to maturity. The photoperiod was 8h.

	Node of first flower																				
TREATMENT	11	12	13	14	15	16	17	18	19	20	22	24	26	28	30	34	38	42	46	50	54
7.5°	4	3	2	0	1	-	1	1	1	2	-	-	-	-	-	-	-	-	-	-	-
20°	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4	3

Fig. 4.1. Mean node of first initiated flower (+S.E.) for intact, decctyledonised (-cots) and grafted plants of lines 58 *(If e sn hr)* and 53 *(If e Sn hr)* either vernalised (V) or unvernalised (UV). Grafting and cotyledon removal were performed when the epicotyl reached a length of 1-2 cm. The photoperiod was 8h.

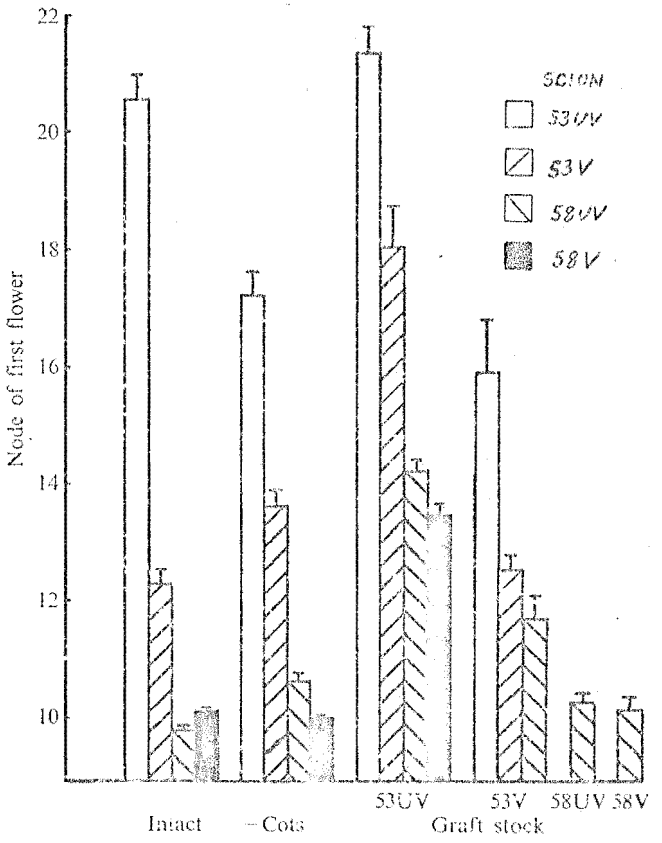

22
20
18
16
14
12
10
Node of first flower
SCION
53UV
53V
58UV
58V
53UV
53V
58UV
58V
Intact
Cots
Graft stock

Table 5.1 Mean node of first initiated flower (FI) ± S.E., flowering time (FT), node with first pod (FP) and total number of leaves expanded (TNE) for lines 68 *(If e sn hr)*, 64 *(If e sn Hr)* and 60 *(If E Sn hr)* exposed to either an 8h photoperiod or continuous light. The plants were either left intact or decotyledonised on day 0 (Embryo) or day 5 (Decot).

		8 Hour Photoperiod						Continuous Light			
		L68		L64		L60		L68		L64	
CHARACTER	TREATMENT	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n	$\bar{X}$ ± S.E.	n
FI	Intact	9.80 ± .11	15	9.87±.13	15	11.13 ±.16	15	9.47±.13	15	10.15±.10	13
FI	Decot	10.36 ± .27	14	14.07±.28	15	18.69 ±.43	13	9.15±.10	13	10.07±.12	15
FI	Embryo	12.87 ± .17	23	16.45±.31	11	16.59 ±.44	17	11.77±.20	13	12.11±.18	18
FT	Intact	34.40 ± .19	15	41.33±.84	15	59.67±2.14	15	29.13±.36	15	30.46±.54	13
FP	Intact	9.80 ± .11	15	12.73±.32	15	18.47 ±.52	15	9.47±.13	15	10.15±.10	13
TNE	Intact	14.40 ± .21	15	20.13±.22	15	23.46 ±.51	13	12.86±.18	14	13.83±.24	12

Table 5.2 The mean node of first initiated flower ± S.E. for lines 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)* and the grafts 53/53, 53/63, 63/53 and 63/63. The photoperiod was 8h and the grafts were performed after 28 days growth (about 9 leaves expanded).

STOCK	SCION				INTACT	
	L53		L63			
	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
L53	18.75± .25	4	20.50± .43	6	20.94± .40	16
L63	20.33± 1.26	6	50.33± 2.11	6	49.88± 1.03	17

Table 5.3 The mean node of first initiated flower ±S.E. for grafts between L53 plants *(lf e Sn hr)* of three ages; young plants (Y) being 5 days old, old plants (O) being 24 days old and flowering plants (F) being 47 days old at the time of grafting. The photoperiod was 8h.

	SCION				INTACT	
	Y		O			
STOCK	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
Y	20.72 ± .29	25	22.86 ± .53	22	22.60 ± .37	10
O	12.86 ± .43	21	20.33 ± .11	18	22.86 ± .59	7
F	11.59 ± .18	23	———		20.60 ± .68	5

Table 5.4 The mean node of first initiated flower (FI)±S.E., number of leaves expanded after 45 days growth (LE)±S.E. and mean adjusted flowering node (Adj. FI) ± S.E. for L53 *(lf e Sn hr)* plants either left intact or defoliated at nodes 6 to 9, 3 to 7 or 3 to 9 on day 26 or defoliated at nodes 6 to 9 on day 26 and at subsequent nodes as they expanded (cont.). The photoperiod was 8h and $n \geq 19$.

CHARACTER	INTACT $\bar{x}$±S.E.	6 to 9 $\bar{x}$±S.E.	3 to 7 $\bar{x}$±S.E.	3 to 9 $\bar{x}$±S.E.	CONT. $\bar{x}$±S.E.
FI	22.83±.35	21.61±.22	23.60±.57	21.42±.32	21.32±.20
LE	17.35±.23	16.43±.22	16.65±.31	14.53±.26	15.56±.15
ADJ. FI	21.93±.43	21.39±.30	23.22±.33	22.61±.57	21.75±.35

Table 5.5 Mean node of first initiated flower ± S.E. for L53 plants exposed to an 8h photoperiod and either left intact or grafted in various ways. The scions were all 4 days old at the time of grafting while the stocks were either 4, 20 or 37 days old (treatments Y, M and O respectively). The grafts were performed either between nodes O and 1 (Y), nodes 5 and 7 (M5 and 05) or nodes 10 and 13 (010) on the stock plants.

05		010		M5		Y		INTACT	
$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
11.71±.36	7	12.00±.26	10	17.27±.53	15	21.43±.37	7	24.00±1.38	11

Table 5.6 L63 plants *(If e Sn Hr)* were either left intact or defoliated at either nodes 3 to 8 (-3 to 8), 5 to 10 or 3 to 10 after 29 days growth. The percentage of plants induced to flower (%) under continuous SD conditions (SD) or after 1,2 or 3 LD cycles (first cycle 32h, then multiples of 24h of light) is indicated along with the number of plants used (n).

	SD		1LD		2LD		3LD	
TREATMENT	%	n	%	n	%	n	%	n
INTACT	0	12	15	13	100	15	100	11
-3 to 8	0	11	0	14	92	12	100	11
-5 to 10	0	11	0	12	27	15	100	14
-3 to 10	0	3	0	7	20	5	40	5

Table 5.7 L63 plants were decotyledonised on day 5(5) or day 27(27). Either 27 day old (young) or 48 day old (old) plants were then exposed to either continuous SD conditions (SD) or 1,2,3,4 or 5 LD cycles (first cycle 32h, then multiples of 24h light). The percentage of plants induced to flower is shown for each treatment (%) as well as the mean number of leaves expanded (LE) at the commencement of the treatment and the number of plants tested (n).

TREATMENT	SD		1LD		2LD		3LD		4LD		5LD		LE
	%	n	%	n	%	n	%	n	%	n	%	n	
YOUNG 27	0	6	25	8	100	11	100	8	100	10	-	-	9.11±.13
OLD 5	0	6	64	11	100	12	100	13	100	12	100	8	12.29±.17
YOUNG 5	0	6	0	10	0	11	25	8	70	10	70	10	6.59±.10

Table 5.8 The mean node of first initiated flower (FI) ± S.E. and number of nodes present when leaf 10 came free of the stiples (TN) ±S.E. for L53 plants (*If e Sn hr*) exposed to an 8h photoperiod and either left intact or decotyledonised on day 5 (Decot 5).

CHARACTER	INTACT		DECOT 5	
	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
FI	23.00 ± .83	12	17.50 ± .43	14
TN	19.17 ± .17	6	17.67 ± .33	6

Table 6.1 This table contains a list of the chemicals tested for their effect on flowering using L61a as the bioassay, the abbreviations used in the text, the reported effect of the chemicals in the literature and references to them.

NAME	ABREVIATION USED (IF ANY)	REPORTED ACTION	REFERENCES
Abscisic acid	ABA	Plant hormone. Inhibitor of active growth.	Addicott & Lyon (1969)
Kinetin	-	Growth regulator. Member of the cytokinin group.	Letham (1967)
Gibberellic acid	GA_3	Plant hormone. Member of the gibberellin group.	Jones (1973)
Ethrel, solution of 2-chloroethylphosphonic acid	-	Breaks down in plants to release ethylene.	Warner & Leopold (1969)
2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidinecarboxylate methyl chloride	AMO1618	Growth retardant. Acts by inhibiting gibberellin synthesis.	McComb & McComb (1970) Cathey (1964)
2-chloroethyletrimethyl-ammonium chloride	CCC	Growth retardant, acts by inhibiting gibberellin synthesis.	Cathey (1964)
Tris-(2-dimethylamine-ethyl)-phosphate trihydrochloride	SK & F7997	Inhibitor of steroid synthesis.	Moore & Anderson (1966)
Androsterone	-	A steroid and acts as a hormone in mammals.	White, Handler & Smith (1967)
Estradiol	-	A steroid and acts as a hormone in mammals.	White, Handler & Smith (1967)
Progesterone	-	A steroid and acts as a hormone in mammals.	White, Handler & Smith (1967)
Cholesterol	-	A steroid found extensively in animals.	White, Handler & Smith (1967)

Table 6.2 The penetrance, mean node of first initiated flower ± S.E. for plants flowering in the early (nodes 10-17) and late regions (nodes 18-34), the length ± S.E. and number of leaves expanded ± S.E. for L61a *(lf e Sn hr)* plants given the various environmental and chemical treatments indicated. Control plants were given an 8 h photoperiod on the trucks, these conditions applying to all other treatments apart from the variable indicated. Vernalisation (V) and continuous light (LD) were given from the start of germination while decotyledonisation (decot) was performed on days 5 and 6. The nodes between which the length measurements were taken and the time at which the number of leaves expanded was recorded varied from experiment to experiment, but provide useful evidence as to the relative vigour of the various treatments within a single experiment.

Experiment	Treatment	Penetrance	Flowering Node				Length	Leaves expanded
			Early		Late			
			$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.
1	EI parents	.68	14.81 ± .44	16	28.03 ± .26	30	14.10 ± .17	
1	L parents	.80	15.00 ± .50	9	27.97 ± .39	38	13.33 ± .16	
1	intact, UV							
1	intact, V	0	12.33 ± .11	46	-	0	14.45 ± .17	
1	decot, UV	1.00	-	0	24.19 ± .23	43	5.31 ± .16	
1	decot, V	-	17.39 ± .14	46	-	0	4.38 ± .08	
2	control	.25	13.38 ± .30	24	26.13 ± .81	8		
2	L53	1.00	-	0	26.50 ± .33	16		
2	LD	0	11.67 ± .14	30	-	0		
2	V	0	11.77 ± .11	30	-	0		
2	decot	1.00	-	0	21.03 ± .20	29		
2	Ethrel	.01	15.60 ± .63	5	25.95 ± .61	22		
3	control	.60	14.25 ± .58	12	25.33 ± .23	18	8.42 ± .14	13.87 ± .16
3	Ethrel .01 mg	.70	14.91 ± .37	11	25.95 ± .32	19	7.38 ± .14	13.90 ± .15
3	Ethrel .1 mg	.83	15.17 ± .40	6	26.33 ± .24	24	6.50 ± .13	13.90 ± .15
3	Ethrel .48 mg	.90	15.33 ± .33	3	27.04 ± .31	26	5.97 ± .13	14.03 ± .13
4	control	.56	14.50 ± .14	14	29.11 ± .36	18	4.30 ± .09	
4	ABA 10 µg	.48	12.57 ± .45	14	28.85 ± .81	13	3.95 ± .12	

Table 6.2 (Continued)

Experiment	Treatment	Penetrance	Flowering node: Early $\bar{x} \pm$ S.E.	Early n	Late $\bar{x} \pm$ S.E.	Late n	Length $\bar{x} \pm$ S.E.	Leaves expanded $\bar{x} \pm$ S.E.
5	control	.72	14.69 ± .30	16	28.93 ± .35	42	3.83 ± .05	
5	ABA 20 ug	.66	13.20 ± .44	10	27.00 ± .67	20	3.24 ± .16	
6	control	.33	13.10 ± .28	20	22.50 ± .37	10	4.50 ± .13	24.00 ± .30
6	Kinetin 20 ppm	.52	12.27 ± .38	11	22.92 ± .40	12	4.67 ± .18	23.78 ± .45
6	Kinetin 100 ppm	.44	11.93 ± .20	14	22.64 ± .58	11	4.55 ± .12	24.04 ± .42
7	control	.25	11.78 ± .26	27	25.33 ± .37	9	7.20 ± .10	9.89 ± .09
7	GA 10 ug	.37	12.19 ± .27	22	26.54 ± .40	13	29.83 ± .79	11.17 ± .13
7	AMO 100 µg	.44	11.83 ± .22	18	23.33 ± .26	14	4.62 ± .08	9.45 ± .10
7	CCC 100 µg	.17	12.07 ± .18	29	24.17 ± .79	6	6.67 ± .11	9.77 ± .08
7	CCC 500 µg	.29	12.21 ± .29	24	25.70 ± .40	10	6.41 ± .10	9.91 ± .09
8	control	.53	12.60 ± .40	15	26.65 ± .31	17	7.57 ± .10	20.84 ± .13
8	Androsterone 1 mg	.70	12.44 ± .18	9	25.32 ± .32	21	7.28 ± .20	21.14 ± .37
8	Cholesterol 1 mg	.70	12.67 ± .37	9	25.76 ± .30	21	7.02 ± .18	21.00 ± .31
8	Estradiol 1 mg	.56	12.21 ± .30	14	26.13 ± .49	16	7.33 ± .15	20.78 ± .37
8	Progesterone 1 mg	.75	12.80 ± .80	5	26.07 ± .26	15	7.20 ± .12	21.19 ± .46
9	control	.87	12.67 ± .21	6	26.34 ± .32	40	5.23 ± .07	22.46 ± .23
9	SKF7997 1000 ppm	1.00		0	25.39 ± .47	20	4.17 ± .14	21.25 ± .33
10	Massey Extract	.89	15.17 ± .48	6	23.14 ± .23	50		
10	Greenfeast Extract	.82	15.40 ± .27	10	22.96 ± .29	45		
11	light seed	.63	13.64 ± .47	11	25.79 ± .42	19	3.70 ± .11	7.73 ± .10
11	heavey seed	.50	14.57 ± .48	14	27.21 ± .66	14	3.73 ± .10	8.64 ± .10
12	Aquasol	.29	13.55 ± .26	22	27.22 ± .88	9	8.05 ± .23	8.09 ± .13
12	Hoaglands	.31	12.55 ± .25	20	28.11 ± .26	9	8.35 ± .11	7.96 ± .20

Table 6.3 Distribution of the node of first initiated flower for L61a plants exposed to an 8 h photoperiod and either given 34 days of vernalisation (V), decotyledonised on day 5 (decot), treated with 480 µg of Ethrel on the dry testa or left untreated (control). A sample of L53 was also grown in an 8 h photoperiod as well as a group of L61a plants exposed to continuous light from the start of germination. The dividing line between penetrant and impenetrant plants was taken as lying between nodes 17 and 18.

Treatment	Node of first flower																						Penetrance
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Control	1	0	6	7	4	4	2	-	-	-	-	1	0	0	0	1	1	3	2	-	-	-	.25
L53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	4	3	5	-	-	-	1.00
LD	1	12	13	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
V	-	9	20	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
decot	-	-	-	-	-	-	-	-	-	2	7	11	6	3	-	-	-	-	-	-	-	-	1.00
Ethrel	-	-	-	1	0	0	3	1	-	-	-	2	0	3	2	2	4	3	2	1	1	2	.81

Table 7.1 The mean node of first initiated flower in L58 *(lf e sn hr)* plants germinated either in vermiculite (controls), on cotton wool in open or closed Petri dishes, or in vermiculite and treated with IAA. The plants were exposed to continuous light.

Experiment 1			Experiment 2		
Treatment	$\bar{x}$ ± S.E.	n	Treatment	$\bar{x}$ ± S.E.	n
Controls	9.72 ± 0.11	18	Controls	10.00 ± 0.10	14
Open Petri dish	10.50 ± 0.27	16	5 µg IAA	9.93 ± 0.07	15
Closed Petri dish	10.83 ± 0.34	18	50 µg IAA	9.79 ± 0.11	14
1 mg IAA	11.40 ± 0.40	10	500 µg IAA	10.42 ± 0.25	14

Table 7.2 The mean node of first initiated flower ±S.E. for lines 58 *(lf e sn hr)*, 53 *(lf e Sn hr)* and 63 *(lf e Sn Hr)* grown under continuous light from the start of germination. The seeds were germinated in Petri dishes on cotton wool soaked in water (C) or aqueous solutions of 2, 20 or 100 ppm of Ethrel.

	L58		L53		L63	
Treatment	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
C	11.53 ± .41	15	12.94 ± .38	18	12.75 ± .37	8
2 ppm	12.08 ± .34	12	14.12 ± .33	17	- -	0
20 ppm	14.33 ± .36	15	14.61 ± .18	18	14.50 ± .57	8
100 ppm	14.92 ± .15	12	15.06 ± .17	18	15.50 ± .50	2

Table 7.3 The mean node of first initiated flower (FI) ± S.E., length between nodes 1 and 6 (L1-6) ± S.E. and the number of leaves expanded after approximately 25 days growth (LE) ± S.E. for lines 58 *(lf e sn hr)*, 59 *(lf E sn hr)*, 64 *(lf e sn Hr)*, 60 *(lf E Sn hr)*, 53 *(lf e Sn hr)* and 51y *(lf sn Hr)*. The plants were treated either once with 0, 24, 96 or 480 µg of Ethrel or watered with a 100p.p.m. solution of Ethrel every week (E). The photoperiod was 8h.

		58		59		64		60		53		51y	
Character	Treatment	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
FI	0	10.06 ± .06	17	9.06 ± .06	17	9.80 ± .11	15	11.00 ± .00	17	20.53 ± .75	17	9.22 ± .10	18
FI	24 µg	12.44 ± .22	18	10.47 ± .12	17	10.82 ± .36	17	12.06 ± .06	17	21.38 ± .50	13	10.59 ± .15	17
FI	96 µg	14.44 ± .22	18	10.94 ± .10	18	12.13 ± .56	15	12.89 ± .21	18	22.94 ± .44	16	11.06 ± .19	18
FI	480 µg	15.25 ± .18	12	11.69 ± .21	13	13.09 ± .51	11	13.38 ± .14	13	23.13 ± .65	15	12.29 ± .19	17
FI	E	15.17 ± .11	12			13.09 ± .67	11						
L1-6	0	7.56 ± .19	17	8.89 ± .26	14	6.38 ± .10	14	5.11 ± .06	16	5.03 ± .15	17	7.28 ± .12	18
L1-6	24 µg	5.79 ± .11	18	7.45 ± .17	16	5.58 ± .17	17	4.76 ± .17	17	5.37 ± .22	14	5.69 ± .13	17
L1-6	96 µg	5.73 ± .16	18	7.64 ± .12	17	5.57 ± .19	15	4.80 ± .15	17	5.56 ± .22	16	5.53 ± .13	18
L1-6	480 µg	4.82 ± .21	12	6.35 ± .17	13	5.42 ± .22	11	4.61 ± .28	13	4.93 ± .18	11	4.85 ± .15	17
L1-6	E	4.83 ± .17	12			5.38 ± .08	10						
LE	0	6.94 ± .06	17	6.19 ± .10	16	7.13 ± .09	15	7.18 ± .10	17	7.24 ± .11	17	7.00 ± .11	18
LE	24 µg	7.78 ± .10	18	6.76 ± .11	17	7.19 ± .16	16	7.82 ± .10	17	7.79 ± .19	14	6.88 ± .12	16
LE	96 µg	7.39 ± .14	14	6.56 ± .13	16	6.67 ± .16	15	7.44 ± .13	16	7.44 ± .13	16	6.83 ± .09	18
LE	480 µg	7.23 ± .17	13	6.69 ± .13	13	6.18 ± .18	11	6.92 ± .08	13	6.73 ± .23	15	6.29 ± .11	17
LE	E	7.08 ± .23	12			6.73 ± .20	11						

Table 7.4 The mean node of first initiated flower (FI)±S.E. and the length between nodes 1 and 6 (L1-6)±S.E. for lines 58 *(lf e sn hr)*, 59 *(lf E sn hr)* and 68 *(lf e sn hr)* treated with either 0 or 480 µg Ethrel. The percent decrease in the internode length caused by Ethrel treatment is also indicated. The plants received continuous light from the time the plumules broke the surface of the growing medium.

Character	Treatment	L58 $\bar{x}$ ± S.E.	n	L59 $\bar{x}$ ± S.E.	n	L68 $\bar{x}$ ± S.E.	n
FI	0	10.29 ± .11	17	9.11 ± .08	18	9.89 ± .08	18
FI	480 µg	13.25 ± .37	8	11.58 ± .15	12	11.56 ± .18	18
L1-6	0	9.82 ± .25	18	11.77 ± .30	17	7.99 ± .23	17
L1-6	480 µg	4.05 ± .16	8	5.06 ± .27	10	4.58 ± .10	18
Percent decrease L1-6		59		52		43	

Table 7.5 The mean node of first initiated flower ± S.E. for L7 plants (*1f^{e} E Sn hr*) either left intact (C) decotyledonised on day 0 (Decot 0) or day 5 (Decot 5) or treated on the cotyledons with 180 µg of Ethrel prior to germination (Ethrel). The photoperiod was 8h.

C		Decot 0		Decot 5		Ethrel	
$\bar{x}\pm$ S.E.	n	$\bar{x}\pm$ S.E.	n	$\bar{x}\pm$ S.E.	n	$\bar{x}\pm$ S.E.	n
$6.33\pm .13$	15	$7.38\pm .11$	21	$6.43\pm .14$	14	$7.36\pm .17$	14

Table 7.6 The percentage of L63 *(f e Sn Hr)* plants induced to flower by 1 long day (LD) cycle (32h of light) after treatment with either 10 µl of ethanol (C) or 10 µl of ethanol containing 480 µg of Ethrel on either the first or second days before the LD cycle, the day of the LD cycle or the first, second or third days after the LD cycles (treatments -1, -2, 0, 1, 2 and 3 respectively). A further group received 480 ug of Ethrel on each of thes 6 days (E). The photoperiod was 8h.

	C	-2	-1	0	1	2	3	E
Percent flowering	89	88	100	93	88	92	100	86
n	19	17	17	14	16	13	12	7

Table 7.7 The table contains the height of the ethylene peak recorded for 1ml samples of air examined using gas chromatography. The air samples came from sealed flasks containing no plants (air), L58 *(1f e sn hr)*, L53 *(1f e Sn hr)* or solutions of 2 or 10 ppm of Ethrel. Both an 8h photoperiod (SD) and continuous light (LD) were used. Three separate runs were carried out, each figure in the table being the mean reading of two air samples for one flask.

Treatment	Run 1	Run 2	Run 3
Air	-	-	.7, .9
L53,SD	4.3, 4.4	1, 1.4	5.4
L58,SD	4.7	2.1, 1.8	5.3
L53,LD	4.0	-	-
Ethrel (2 ppm)	-	-	26.5
Ethrel (10 ppm)	-	-	52.0

Fig. 7.1 Effect of Ethrel on the mean node of first initiated flower of L58 *(lf e sn hr)* plants germinated in open Petri dishes on cotton wool. Vertical bars indicate twice the standard errors; n = 18. The plants were exposed to continuous light.

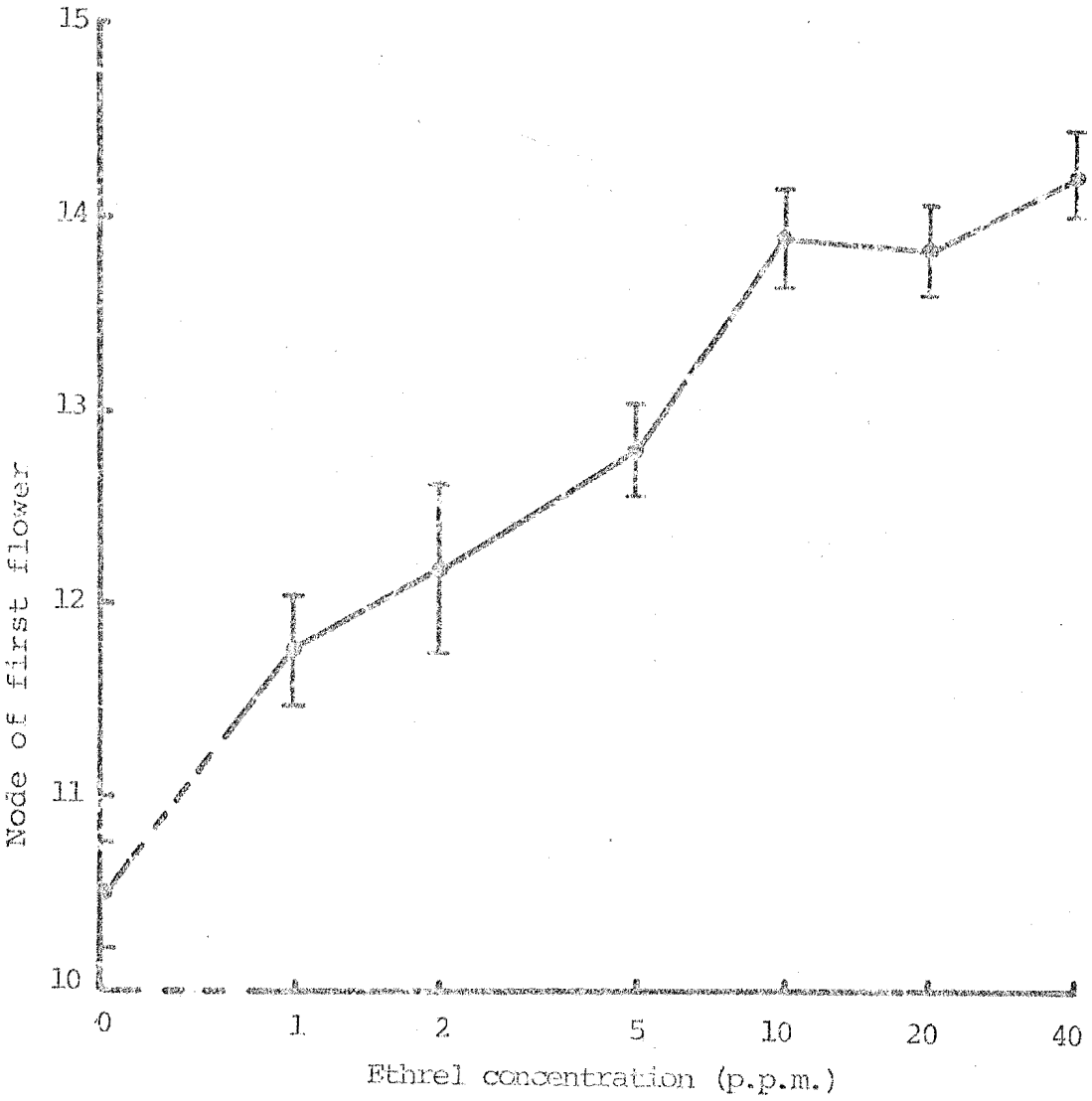

15
14
13
12
11
10
Node of first flower
0
1
2
5
10
20
40
Ethrel concentration (p.p.m.)

Fig. 7.2 Regression of the mean flowering node of progeny of L64 plants *(If e sn Hr)* plotted against the flowering node of the parent ($y = 0.50x + 7.53$). Both the parents and progeny were exposed to an 8h photoperiod and were treated with 480 µg of Ethrel on the cotyledons prior to germination. The slope of the regression is significantly different from 0 (at the 0.05 level).

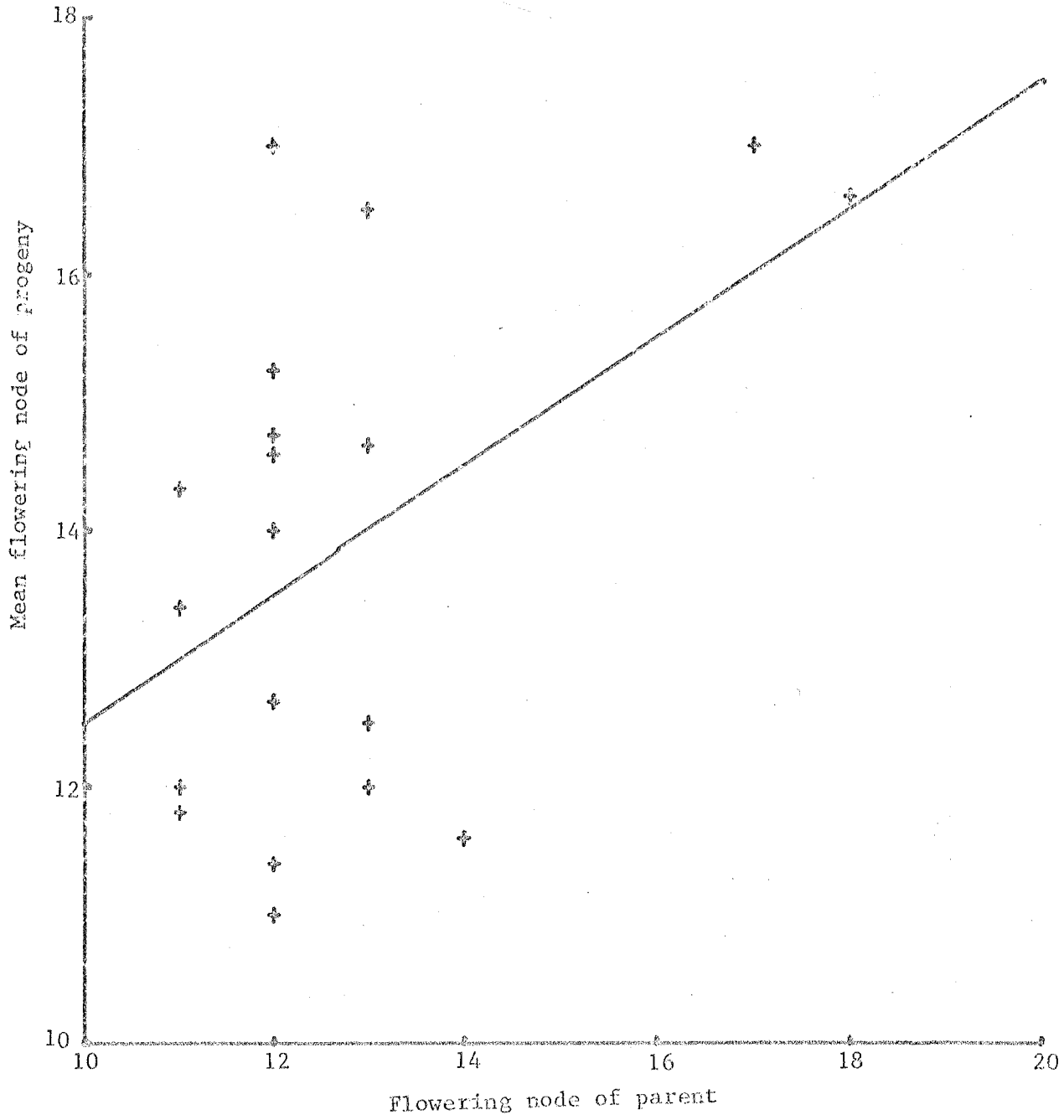

18
16
14
12
10
Mean flowering node of progeny
10
12
14
16
18
20
Flowering node of parent

Table 8.1 The mean node of first initiated flower (FI) ± S.E., number of days to the first open flower (FT) ± S.E., node of first open flower (FD) ± S.E. and total number of leaves expanded by the plant (TNE) ± S.E., for L24 plants *(Lf e Sn hr)* exposed to either continuous light or an 8h photoperiod. The plants received no GA_3 (Control), a single 10 µg dose of GA_3 prior to imbibition (Day 0) or a 10 µg dose of GA_3 every two weeks (Continuous). The smallest number of plants scored was sixteen. Significance levels indicate differences from the respective control treatments.

CHARACTER	Continuous Light			8h Light		
	CONTROL $\bar{x}\pm$S.E.	DAY 0 $\bar{x}\pm$S.E.	CONTINUOUS $\bar{x}\pm$S.E.	CONTROL $\bar{x}\pm$S.E.	DAY 0 $\bar{x}\pm$S.E.	CONTINUOUS $\bar{x}\pm$S.E.
FI	17.18±.16	17.71±.11^{x}	17.56±.16	24.64±.33	29.75±.57^{xxx}	32.06±.45^{xxx}
FT	60.65±.31	58.35±.41^{xxx}	60.61±.78	88.24±.87	90.44±.61^{x}	89.44±.61
FD	17.18±.16	17.71±.11^{x}	18.72±.27^{xxx}	24.64±.33	29.75±.57^{xxx}	32.06±.45^{xxx}
TNE	18.47±.23	19.65±.23^{xx}	21.89±.31^{xxx}	25.59±.32	30.81±.65^{xxx}	32.90±.47^{xxx}

Table 8.2 The mean node of first initiated flower (FI) ±S.E.,number of leaves expanded (LE) on days 29, 39 and 55 ±S.E., and length between nodes 1 and 6(L1-6), 6 and 10(L6-10), 10 and 14(10-14) and 14 and 20(L14-20) for L24 plants *(Lf e Sn hr)* exposed to an 8h photoperiod. The plants either received no GA_3 (Control), 10µg of GA_3 on either day 0, 29 or 39 or 10µg of GA_3 every 2 weeks (Continuous). The smallest number of plants scored was 20. Significance levels indicate differences from the relevant control treatment.

CHARACTER	FI	LE on day 29	LE on day 39	LE on day 55	L1-6	L6-10	L10-14	L14-20
TREATMENT	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.
CONTROL	26.40 ± .48	9.10 ± .07	12.95 ± .09	19.10 ± .14	5.81 ± .11	8.10 ± .21	8.30 ± .17	16.49 ± .43
DAY 0	30.17 ± .42***	10.30 ± .17***	14.14 ± .18***	19.91 ± .26**	30.33 ± 1.20***	11.68 ± .83***	7.70 ± .23*	13.17 ± .43***
DAY 29	27.96 ± .49*	8.68 ± .12**	13.23 ± .20	20.76 ± .28***	5.92 ± .20	13.53 ± .89***	37.20 ± .75***	50.49 ± 2.50***
DAY 39	27.00 ± .35	9.10 ± .07	12.81 ± .13	19.95 ± .22**	5.77 ± .10	8.07 ± .31	12.60 ± .68***	63.27 ± 1.05***
CONTINUOUS	33.70 ± .32***	11.00 ± .09***	15.88 ± .17***	22.95 ± .20***	29.34 ± 1.20***	29.44 ± .96***	20.86 ± .66***	44.11 ± 1.55***

Table 8.3 The mean node of first initiated flower (FI) ± S.E., number of days to first open flower (FT) ± S.E., node of first pod (FP) ±S.E. and first node with more than 2 leaflets (L>2) ± S.E. for lines 63 *(lf e Sn Hr)* and 53 *(lf e Sn hr)*. The plants were either left untreated (Control) or treated with GA_3 and either exposed to an 8h photoperiod (SD) or to continuous light (LD). The smallest number of plants scored was 16. Significance levels indicate differences from the relevant control treatment.

	L53				L63			
	LD		SD		LD		SD	
Character	Control	GA_3	Control	GA_3	Control	GA_3	Control	GA_3
	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.	$\bar{x}$ ± S.E.
FI	14.22 ± .14	13.38 ± .23**	21.28 ± .37	24.24 ± .53***	14.65 ± .19	13.63 ± .22**	44.69 ± .69	44.87 ± .95
FT	34.95 ± .44	32.24 ± .30***	61.88 ± 1.19	60.00 ± .98	37.26 ± .42	36.87 ± .62	-	-
FP	14.26 ± .14	13.95 ± .22	22.21 ± .37	24.84 ± .65**	14.65 ± .19	14.44 ± .33	-	-
L>2	12.00 ± .27	13.95 ± .35***	16.17 ± .60	22.26 ± .51***	15.09 ± .32	16.63 ± .24**	18.56 ± .72	24.06 ± .73***

Table 8.4 The mean node of first initiated flower ± S.E. for lines 64 *(lf e sn Hr)* and 68 *(lf e sn hr)* exposed to either continuous light (LD) or an 8h photoperiod (SD) and either left intact or decotyledonised on days 6 and 7 (-). Plants were treated with either 10 µg of GA_3, 100 µg of AMO 1618 or 1000 µg of CCC prior to imbibition or left untreated (Control). Significance levels indicate differences between the various chemical treatments and the relevant control treatment.

Treatment	Control		GA		AMO1618		CCC	
	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n	$\bar{x}$ ± S.E.	n
L64,SD, INTACT	9.95 ± .12	19	12.40 ± .81 ***	5	9.94 ± .25	16	10.06 ± .10	18
L64,SD, -	11.84 ± .65	19	16.33 ± .41 ***	12	12.06 ± .47	18	13.16 ± .79	19
L64,LD, INTACT	9.95 ± .19	19	11.07 ± .22 ***	14	-		-	
L64,LD,-	9.94 ± .06	18	11.42 ± .19 ***	12	-		-	
L68,SD, INTACT	10.41 ± .15	17	10.90 ± .31	10	10.24 ± .18	17	10.13 ± .18	16
L68,SD,-	11.38 ± .27	16	11.50 ± .22	10	10.95 ± .24	19	10.79 ± .14	21
L68,LD, INTACT	10.26 ± .15	15	11.18 ± .38 *	11	-		-	
L68,LD, -	10.20 ± .12	20	11.29 ± .24 ***	14	-		-	

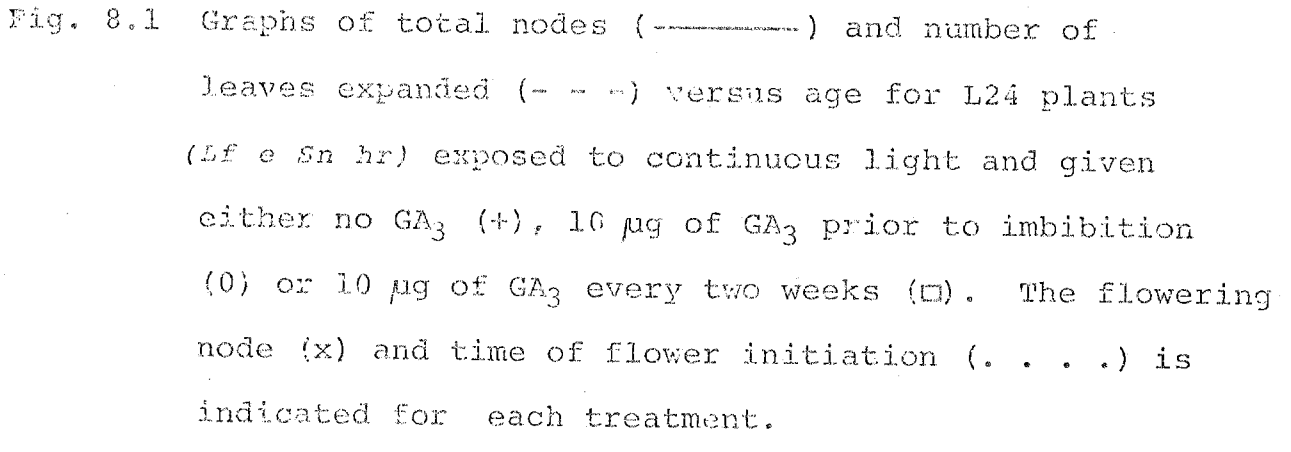


Fig. 8.1 Graphs of total nodes (————) and number of leaves expanded (- - -) versus age for L24 plants (*Lf e Sn hr*) exposed to continuous light and given either no GA_3 (+), 10 µg of GA_3 prior to imbibition (0) or 10 µg of GA_3 every two weeks (□). The flowering node (x) and time of flower initiation (. . . .) is indicated for each treatment.

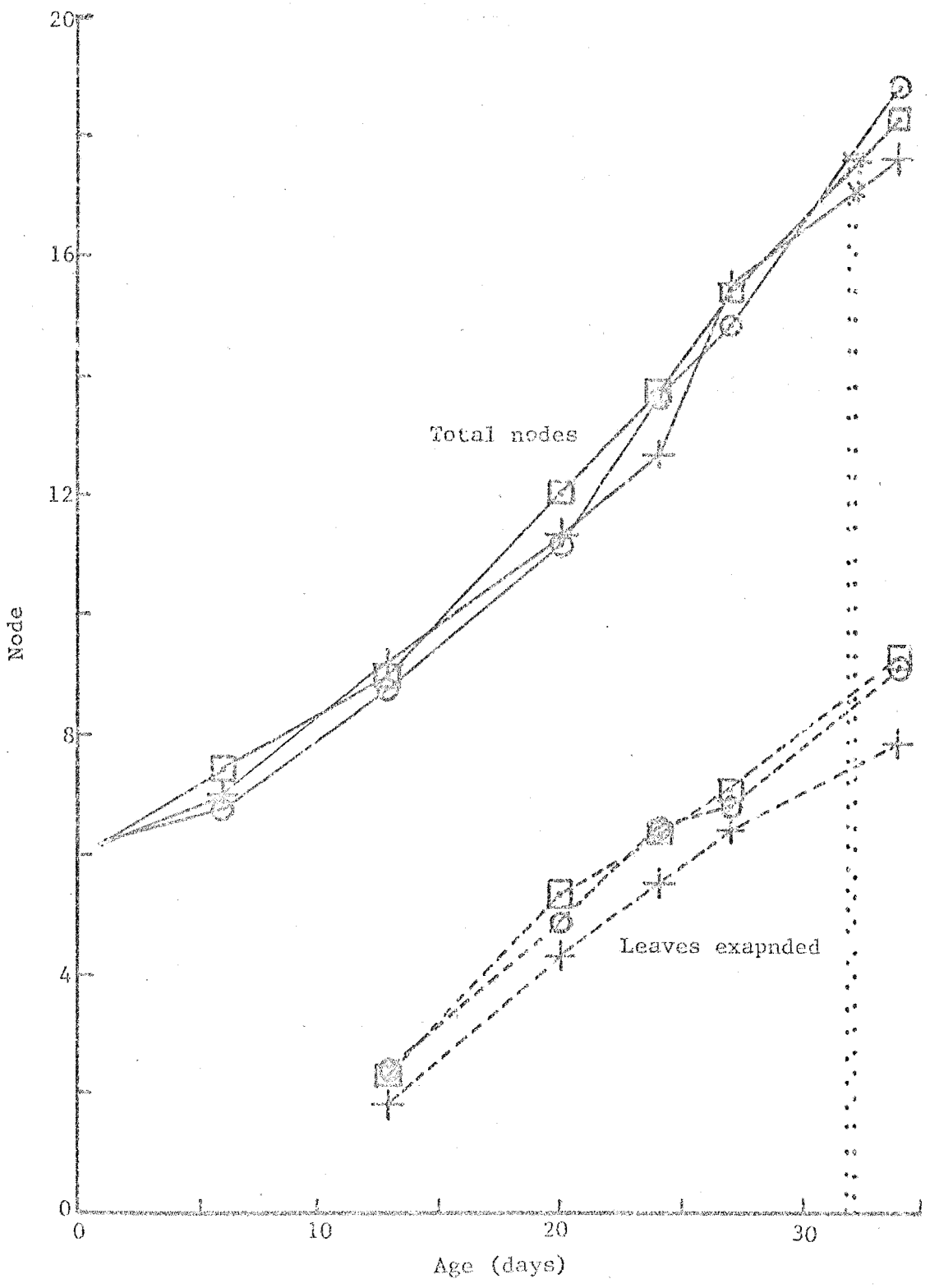

20
16
12
8
4
0
Node
Total nodes
Leaves exapnded
0
10
20
30
Age (days)

Fig. 8.2 Graphs of total nodes (————) and number of leaves expanded (- - -) versus age for L24 plants *(Lf e Sn hr)* exposed to an 8h photoperiod and given either no GA_3 (+), 10 µg of GA_3 prior to germination (0) or 10 µg of GA_3 every two weeks (□). The flowering node (x) and time of flower initiation (. . . .) is indicated for each treatment.

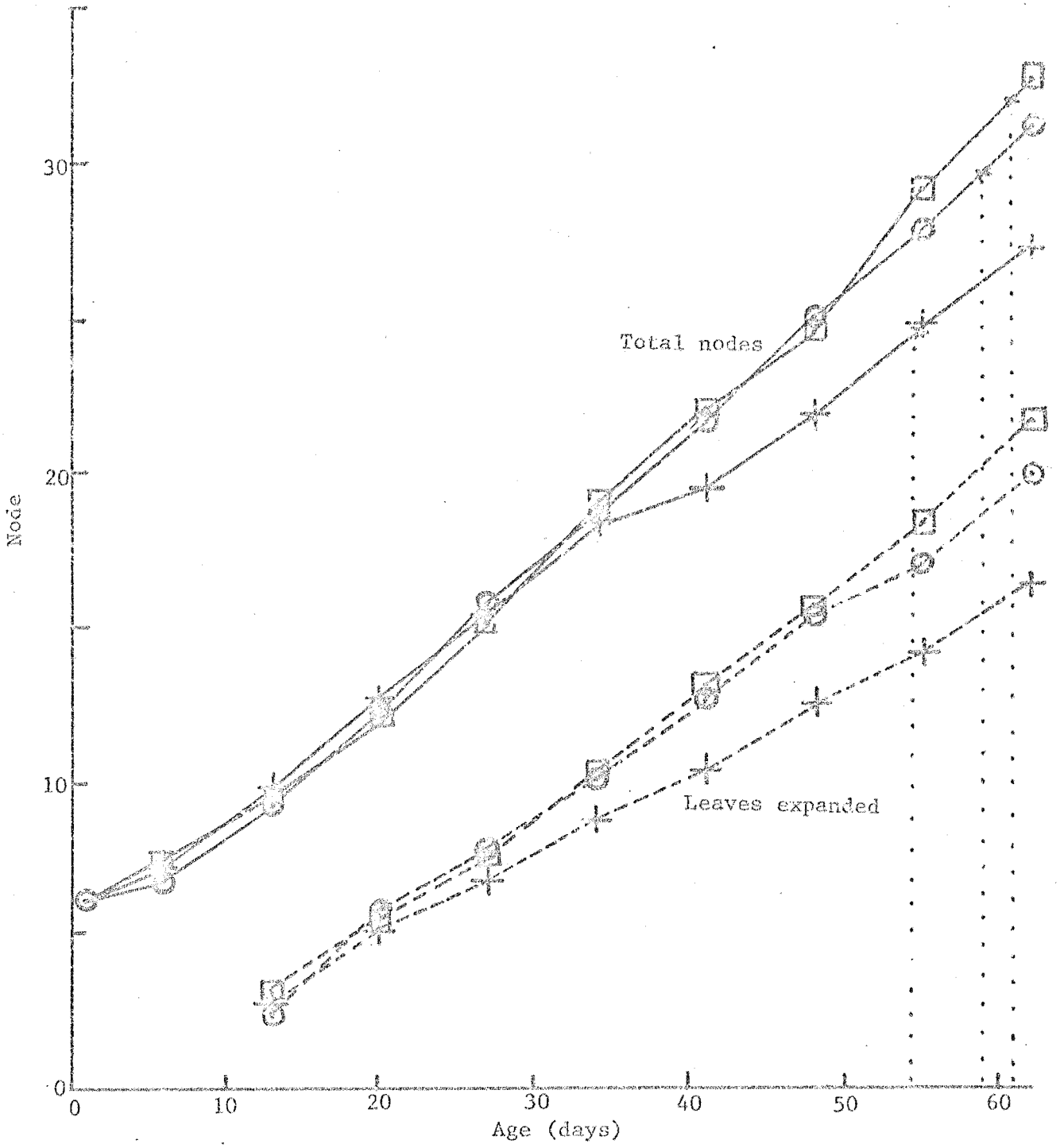

Node
30
20
10
0
0
10
20
30
40
50
60
Age (days)
Total nodes
Leaves expanded

Fig. 9.1 The proposed variation in the ratio of promoter to inhibitor with age is shown for various genotypes at the *e*, *sn* and *hr* loci exposed to either an 8h photoperiod (SD) or continuous light (LD). Arbitary thresholds controlled by alleles at the *lf* locus are shown. The effect of cotyledon removal on day 5 (-) and exposure of the cotyledons to the photoperiod from the start of germination (ex) are indicated where relevant, as well as intact plants (+) and plants with their cotyledons buried (bur.). A mean temperature of 20°C is assumed.

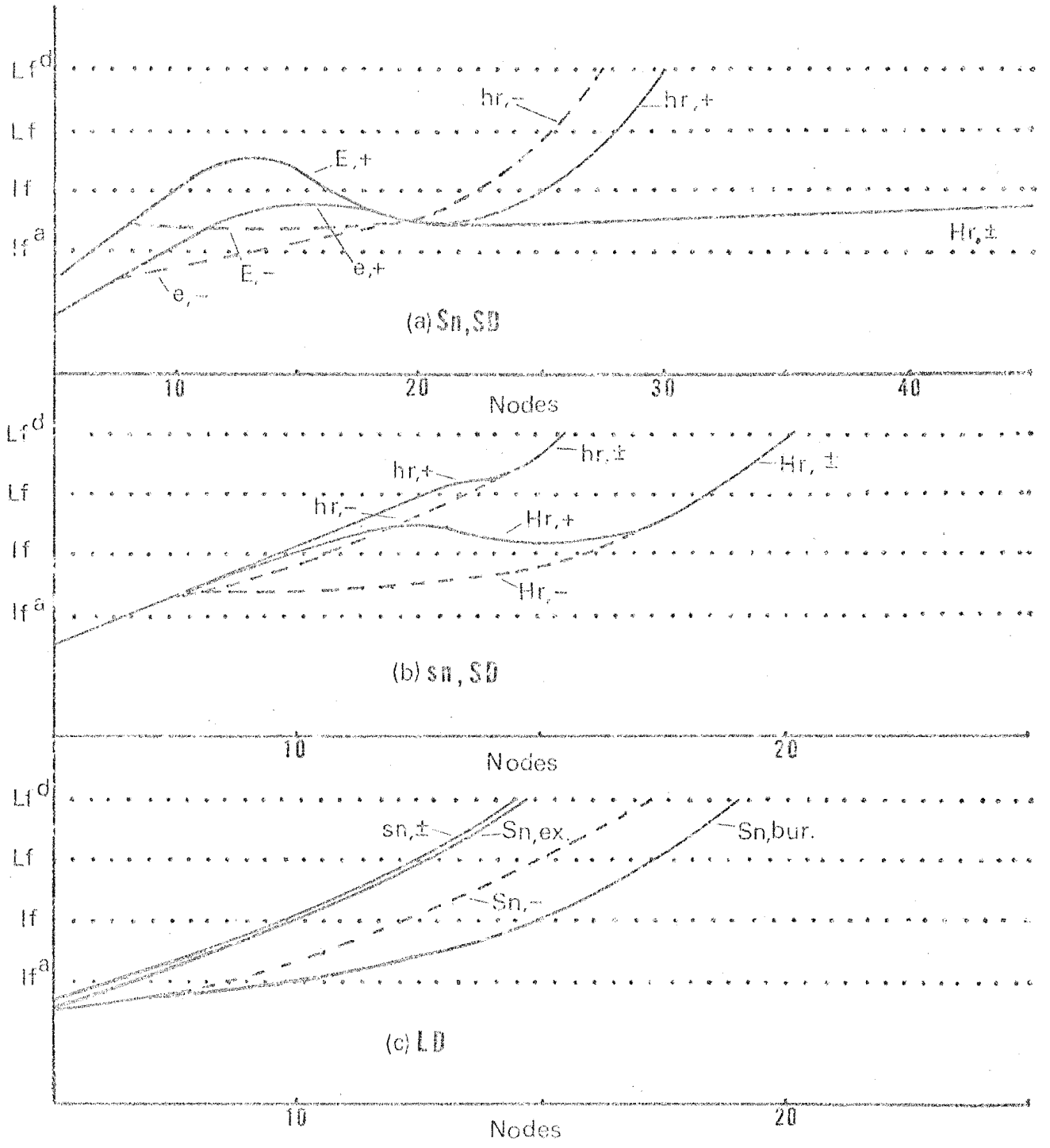
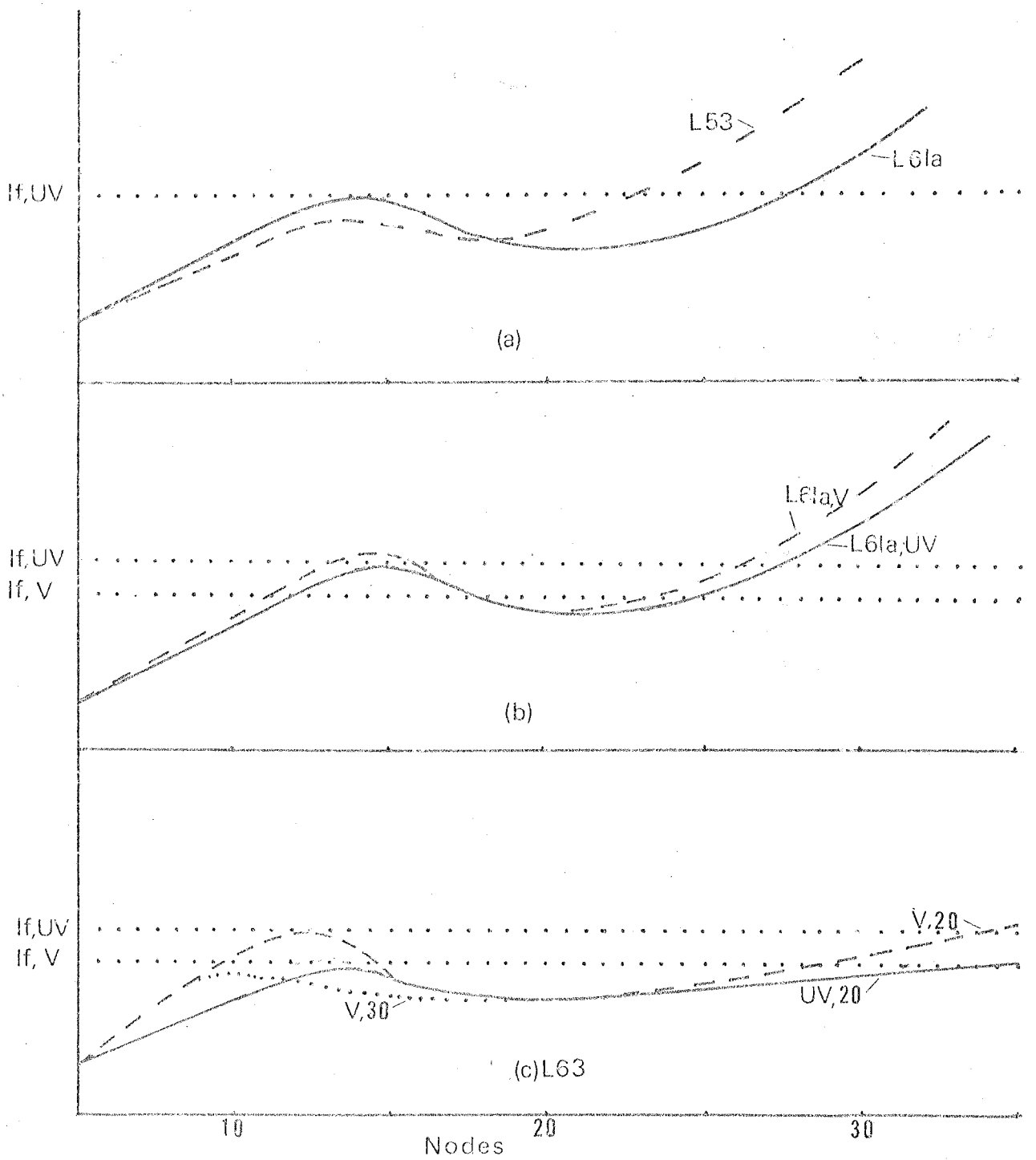

Lf^d
Lf
If
If^a
hr,−
hr,+
E,+
E,−
e,+
e,−
Hr,±
(a) Sn, SD
10
20
30
40
Nodes
Lf^d
Lf
If
If^a
hr,+
hr,±
hr,−
Hr,+
Hr,−
Hr,±
(b) sn, SD
10
20
Nodes
Lf^d
Lf
If
If^a
sn,±
Sn,ex.
Sn,bur.
Sn,−
(c) LD
10
20
Nodes

Fig. 9.2 The proposed variation in the ratio of promotor to inhibitor with age for lines 61a *(1f e Sn hr)*, 53 *(1f e Sn hr)* and 63 *(1f e Sn Hr)* and given either vernalisation (V), vernalisation followed by treatment at 30°C (V,30) or no vernalisation (UV). At other times a temperature of 20°C and a photoperiod of 8h is assumed.


L53
L6la
If,UV
(a)
L6la,V
L6la,UV
If,UV
If, V
(b)
V,20
If,UV
If, V
V,30
UV,20
(c)L63
10
20
30
Nodes